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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

DECLARATION OF TOD BEDILION, Ph.D.
UNDER 37 C.F.R. § 1.132

I, TOD BEDILION, Ph.D., declare and state as follows:

1. In April, 1996, I became the first employee of Synteni, Inc., where I served as Research Director until its acquisition by Incyte Corporation in early 1998. After Synteni's acquisition, I continued in the position of Director of Corporate Development at Incyte until May 11, 2001. I am currently the Director of Business Development at Genomic Health, Inc., Redwood City, California and an occasional Consultant to Incyte.

2. Synteni was founded to commercialize expression microarrays, microarrays in which expressed nucleic acids -- full-length cDNAs, fragments of full-length cDNAs, expressed sequence tags (ESTs) -- are arrayed on a common support to permit highly parallel detection and measurement of the expression of their cognate genes in a biological sample.

3. During my employ at Synteni, virtually all (if not all) of my work efforts were directed to the further technical development and the commercial exploitation of that microarray technology; given the small size of our shop, most of us had both technical and commercial responsibilities. The customer accounts for which I was personally responsible included large pharmaceutical companies, such as SmithKline

Beecham, large biotechnology companies, such as Genentech, and small research institutes, such as DNAX Inc.

4. From my very first interaction with our customers, consistently through to Synteni's acquisition by Incyte, I heard uniform, consistent, and emphatic requests that more genes be added to the arrays. This was true with respect to both our original microarrays, based on customer-provided genes and libraries, and our later, "generic", gene expression microarrays, based upon the unigene clone collection (our so-called "UniGem" arrays). From day 1, the pressure on us was to print ever more spots on the array. It was never a question: our customers wanted ever more genes on the array, each new gene-specific probe providing incrementally more value to the customer.¹

5. As a commercial enterprise, providing value to our customers was our major concern. Thus, to increase the value of our products and services in the marketplace -- to increase our ability to sell our microarrays and microarray services, their "salability" -- our efforts from the very beginning were devoted to increasing the number of specific genes whose expression could be detected with our microarrays.

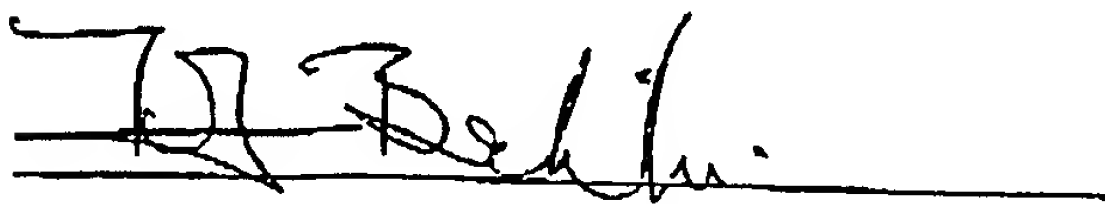
6. Indeed, one of our major competitive advantages in the marketplace -- not just as regards other commercial suppliers, but also with respect to the innumerable laboratories and companies that were attempting to spot arrays in their own "home-brew" facilities -- was the number of

¹ I should note the customers were not asking for addition of probes specific to only those genes for which the biological function of the encoded gene product was known, but were asking for probes specific to any and all expressed genes.

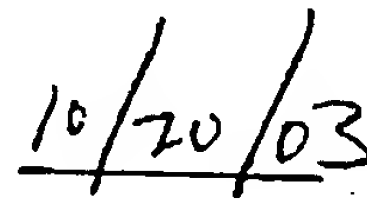
distinct gene-specific probes that we provided on our expression microarrays. Our first 10,000 element UniGem array put the holy grail of gene expression analysis -- the human whole genome array -- within sight for the very first time (with respect to timing of the UniGEM program we began project planning and technology development in mid 1996 and delivered our first 10,000 element standard content human arrays in the first months of 1997 as I recall).

7. By the end of 1997, our efforts to provide the most comprehensive, and thus most valuable, human gene expression microarrays had been sufficiently successful that Incyte agreed to acquire Synteni for a reported \$80 million.

8. I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and may jeopardize the validity of any patent application in which this declaration is filed or any patent that issues thereon.



Tod Bedilion, Ph.D.



Date

PCT

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(21) International Application Number: PCT/US95/01863 (22) International Filing Date: 14 February 1995 (14.02.95) (30) Priority Data: 08/195,485 14 February 1994 (14.02.94) US (60) Parent Application or Grant (63) Related by Continuation US 08/195,485 (CIP) Filed on 14 February 1994 (14.02.94) (71) Applicant (for all designated States except US): SMITHKLINE BEECHAM CORPORATION [US/US]; Corporate Intellectual Property, UW2220, 709 Swedeland Road, P.O. Box 1539, King of Prussia, PA 19406-0939 (US). (72) Inventors; and (75) Inventors/Applicants (for US only): ROSENBERG, Martin [US/US]; 241 Mingo Road, Royersford, PA 19468 (US). DEBOUCK, Christine [BE/US]; 667 Pugh Road, Wayne, PA 19087 (US). BERGSMA, Derk [US/US]; 271 Irish Road, Berwyn, PA 19312 (US).		(74) Agents: JERVIS, Herbert, H. et al.; SmithKline Beecham Corporation, Corporate Intellectual Property, UW2220, 709 Swedeland Road, P.O. Box 1539, King of Prussia, PA 19406-0939 (US). (81) Designated States: JP, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published <i>With international search report.</i>
(54) Title: DIFFERENTIALLY EXPRESSED GENES IN HEALTHY AND DISEASED SUBJECTS (57) Abstract The present invention involves methods and compositions for identifying genes which are differentially expressed in a normal healthy animal and an animal having a selected disease or infection, and methods for diagnosing diseases or infections characterized by the presence of those genes, despite the absence of knowledge about the gene or its function. The methods involve the use of a composition suitable for use in hybridization which consists of a solid surface on which is immobilized at pre-defined regions thereon a plurality of defined oligonucleotide/polynucleotide sequences for hybridization. Each sequence comprises a fragment of an EST isolated from an identified DNA library prepared from tissue or cell samples of a healthy animal, an animal with a selected disease or infection, and any combination thereof. Differences in hybridization patterns produced through use of this composition and the specified methods enable diagnosis of disease based on differential expression of genes of unknown function, and enable the identification of those genes and the proteins encoded thereby.		

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differentially expressed genes in healthy and diseased subjects

Cross Reference to Related Applications:

5 This application is a continuation-in-part application of U.S. Serial No. 08/195,485 filed February 14, 1994, the contents of which are incorporated herein by reference.

Field of the Invention

10 The present invention relates to the use of immobilized oligonucleotide/polynucleotide or polynucleotide sequences for the identification, sequencing and characterization of genes which are implicated in disease, infection, or development and the use of such identified genes and the proteins encoded thereby in diagnosis, prognosis, therapy and drug discovery.

15

Background of the Invention

 Identification, sequencing and characterization of genes, especially human genes, is a major goal of modern scientific research. By identifying genes, determining their sequences and characterizing their biological function, it is possible
20 to employ recombinant DNA technology to produce large quantities of valuable "gene products", e.g., proteins and peptides. Additionally, knowledge of gene sequences can provide a key to diagnosis, prognosis and treatment of a variety of disease states in plants and animals which are characterized by inappropriate expression and/or repression of selected gene(s) or by the influence of external factors, e.g., carcinogens
25 or teratogens, on gene function. The term disease-associated genes(s) is used herein in its broadest sense to mean not only genes associated with classical inherited diseases, but also those associated with genetic predisposition to disease as well as infectious or pathogenic states resulting from gene expression by infectious agents or the effect on host cell gene expression by the presence of such a pathogen or its
30 products. Locating disease-associated genes will permit the development of diagnostic and prognostic reagents and methods, as well as possible therapeutic regimens, and the discovery of new drugs for treating or preventing the occurrence of such diseases.

 Methods have been described for the identification of certain novel
35 gene sequences, referred to as Expressed Sequence Tags (EST) [see, e.g., Adams et al, Science, 252:1651-1656 (1991); and International Patent Application No. WO93/00353, published January 7, 1993]. Conventionally, an EST is a specific cDNA polynucleotide sequence, or tag, about 150 to 400 nucleotides in length, derived from

a messenger RNA molecule by reverse transcription, which is a marker for, and component of, a human gene actually transcribed *in vivo*. However, as used herein an EST also refers to a genomic DNA fragment derived from an organism, such as a microorganism, the DNA of which lacks intron regions.

5 A variety of techniques have been described for identifying particular gene sequences on the basis of their gene products. For example, several techniques are described in the art [see, e.g., International Patent Application No. WO91/07087, published May 30, 1991]. Additionally, known methods exist for the amplification of desired sequences [see, e.g., International Patent Application No. WO91/17271,
10 published November 14, 1991, among others].

 However, at present, there exist no established methods for filling the need in the art for methods and reagents which employ fragments of differentially expressed genes of known, unknown (or previously unrecognized) function or consequence to provide diagnostic and therapeutic methods and reagents for diagnosis
15 and treatment of disease or infection, which conditions are characterized by such genes and gene products. It should be appreciated that it is the expression differences that are diagnostic of the altered state (e.g., predisease, disease, pathogenic, progression or infectious). Such genes associated with the altered state are likely to be the targets of drug discovery, whether the genes are the cause or the effect of the
20 condition, identification of such genes provides insight into which gene expression needs to be re-altered in order to reestablished the healthy state.

Summary of the Invention

 In one aspect, the invention provides methods for identifying gene(s)
25 which are differentially expressed, for example, in a normal healthy organism and an organism having a disease. The method involves producing and comparing hybridization patterns formed between samples of expressed mRNA or cDNA polynucleotide sequences obtained from either analogous cells, tissues or organs of a healthy organism and a diseased organism and a defined set of
30 oligonucleotide/polynucleotide/polynucleotide sequence probes from either an healthy organism or a diseased organism immobilized on a support. Those defined oligonucleotide/polynucleotide sequences are representative of the total expressed genetic component of the cells, tissues, organs or organism as defined the collection of partial cDNA sequences (ESTs). The differences between the hybridization
35 patterns permit identification of those particular EST or gene-specific oligonucleotide/polynucleotide sequences associated with differential expression, and the identification of the EST permits identification of the clone from which it was

derived and using ordinary skill further cloning and, if desired, sequencing of the full-length cDNA and genomic counterpart, i.e., gene, from which it was obtained.

5 In another aspect, the invention provides methods substantially similar to those described above, but which permit identification of those gene(s) of a pathogen which are expressed in any biological sample of an infected organism based on comparative hybridization of RNA/cDNA samples derived from a healthy versus infected organism, hybridized to an oligonucleotide/polynucleotide set representative of the gene coding complement of the pathogen of interest.

10 In another aspect, the invention provides methods substantially similar to those described above, but which permit identification of those ESTs-specific oligonucleotide/polynucleotide sequences of host gene(s) which represent genes being differentially expressed/ altered in expression by the disease state, or infection and are expressed in any biological sample of an infected organism based on comparative hybridization of RNA/cDNA samples derived from a healthy versus infected
15 organism of interest.

In a further aspect, the methods described above and in detail below, also provide methods for diagnosis of diseases or infections characterized by differentially expressed genes, the expression of which has been altered as a result of infection by the pathogen or disease causing agent in question. All identified
20 differences provide the basis for diagnostic testing be it the altered expression of endogenous genes or the patterned expression of the genes of the infecting organism. Such patterns of altered expression are defined by comparing RNA/cDNA from the two states hybridized against a panel of oligonucleotide/polynucleotides representing the expressed gene component of a cell, tissue, organ or organism as defined by its
25 collection of ESTs.

Yet a further aspect of this invention provides a composition suitable for use in hybridization, which comprises a solid surface on which is immobilized at pre-defined regions thereon a plurality of defined oligonucleotide/polynucleotide sequences for hybridization, each sequence comprising a fragment of an EST isolated
30 from a cDNA or DNA library prepared from at least one selected tissue or cell sample of a healthy (i.e., pre-disease state) animal, at least one analogous sample of an animal having a disease, at least one analogous sample of an animal infected with a pathogen or the pathogen itself, or any combination or multiple combinations thereof.

An additional aspect of the invention provides an isolated gene
35 sequence which is differentially expressed in a normal healthy animal and an animal having a disease, and is identified by the methods above. Similarly, an isolated pathogen gene sequence which is expressed in tissue or cell samples of an infected animal can be identified by the methods above.

Yet another aspect of the invention is that it provides not only a means for a static diagnostic but also provides a means for a carrying out the procedure over time to measure disease progression as well as monitoring the efficacy of disease treatment regimes including an toxicological effects thereof.

5 Another aspect of the invention is an isolated protein produced by expression of the gene sequences identified above. Such proteins are useful in therapeutic compositions or diagnostic compositions, or as targets for drug development.

10 Other aspects and advantages of the present invention are described further in the following detailed description of the preferred embodiments thereof.

Detailed Description of the Invention

15 The present invention meets the unfulfilled needs in the art by providing methods for the identification and use of gene fragments and genes, even those of unknown full length sequence and unknown function, which are differentially expressed in a healthy animal and in an animal having a specific disease or infection by use of ESTs derived from DNA libraries of healthy and/or diseased/infected animals. Employing the methods of this invention permits the resulting identification and isolation of such genes by using their corresponding ESTs
20 and thereby also permits the production of protein products encoded by such genes. The genes themselves and/or protein products, if desired, may be employed in the diagnosis or therapy of the disease or infection with which the genes are associated and in the development of new drugs therefor.

25 It has been appreciated that one or more differentially identified EST or gene-specific oligonucleotide/polynucleotides define a pattern of differentially expressed genes diagnostic of a predisease, disease or infective state. A knowledge of the specific biological function of the EST is not required only that the ESTs identifies a gene or genes whose altered expression is associated reproducibly with the predisease, disease or infectious state. The differences permit the identification of
30 gene products altered in their expression by the disease and represent those products most likely to be targets of therapeutic intervention. Similarly, the product may be of the infecting organism itself and also be an effective target of intervention.

1. Definitions.

35 Several words and phrases used throughout this specification are defined as follows:

As used herein, the term "gene" refers to the genomic nucleotide sequence from which a cDNA sequence is derived, which cDNA produces an EST, as

described below. The term gene classically refers to the genomic sequence, which, upon processing, can produce different cDNAs, e.g., by splicing events. However, for ease of reading, any full-length counterpart cDNA sequence which gives rise to an EST will also be referred to by shorthand herein as a 'gene'.

5 The term "organism" includes without limitation, microbes, plants and animals.

 The term "animal" is used in its broadest sense to include all members of the animal kingdom, including humans. It should be understood, however, that according to this invention the same species of animal which provides the biological
10 sample also is the source of the defined immobilized oligonucleotide/polynucleotides as defined below.

 The term "pathogen" is defined herein as any molecule or organism which is capable of infecting an animal or plant and replicating its nucleic acid sequences in the cells or tissues of that animal or plant. Such a pathogen is generally
15 associated with a disease condition in the infected animal or plant. Such pathogens may include viruses, which replicate intra- or extra-cellularly, or other organisms, such as bacteria, fungi or parasites, which generally infect tissues or the blood. Certain pathogens or microorganisms are known to exist in sequential and distinguishable stages of development, e.g., latent stages, infective stages, and stages
20 which cause symptomatic diseases. In these different stages, the pathogens are anticipated to express differentially certain genes and/or turn on or off host cell gene expression.

 As used herein, the term "disease" or "disease state" refers to any condition which deviates from a normal or standardized healthy state in an organism
25 of the same species in terms of differential expression of the organism's genes. In other words, a disease state can be any illness or disorder be it of genetic or environmental origin, for example, an inherited disorder such as certain breast cancers, or a disorder which is characterized by expression of gene(s) normally in an inactive, 'turned off' state in a healthy animal, or a disorder which is characterized by
30 under-expression or no expression of gene(s) which is normally activated or 'turned on' in a normal healthy animal. Such differential expression of genes may also be detected in a condition caused by infection, inflammation, or allergy, a condition caused by development or aging of the animal, a condition caused by administration of a drug or exposure of the animal to another agent, e.g., nutrition, which affects
35 gene expression. Essentially, the methods described herein can be adapted to detect differential gene expression resulting from any cause, by manipulation of the defined oligonucleotide/polynucleotides and the samples tested as described below. The

concept of disease or disease state also includes its temporal aspects in terms of progression and treatment.

The phrase "differentially expressed" refers to those situations in which a gene transcript is found in differing numbers of copies, or in activated vs inactivated states, in different cell types or tissue types of an organism, having a selected disease as contrasted to the levels of the gene transcript found in the same cells or tissues of a healthy organism. Genes may be differentially expressed in differing states of activation in microorganisms or pathogens in different stages of development. For example, multiple copies of gene transcripts may be found in an organism having a selected disease, while only one, or significantly fewer copies, of the same gene transcript are found in a healthy organism, or vice-versa.

As used herein, the term "solid support" refers to any known substrate which is useful for the immobilization of large numbers of oligonucleotide/polynucleotide sequences by any available method to enable detectable hybridization of the immobilized oligonucleotide/polynucleotide sequences with other polynucleotide sequences in a sample. Among a number of available solid supports, one desirable example is the supports described in International Patent Application No. WO91/07087, published May 30, 1991. Also useful are supports such as but not limited to nitrocellulose, myelin, glass, silica and Pall Biodyne C®. It is also anticipated that improvements yet to be made to conventional solid supports may also be employed in this invention.

The term "surface" means any generally two-dimensional structure on a solid support to which the desired oligonucleotide/polynucleotide sequence is attached or immobilized. A surface may have steps, ridges, kinks, terraces and the like.

As used herein, the term "predefined region" refers to a localized area on a surface of a solid support on which is immobilized one or multiple copies of a particular oligonucleotide/polynucleotide sequence and which enables the identification of the oligonucleotide/polynucleotide at the position, if hybridization of that oligonucleotide/polynucleotide to a sample polynucleotide occurs.

By "immobilized" refers to the attachment of the oligonucleotide/polynucleotide to the solid support. Means of immobilization are known and conventional to those of skill in the art, and may depend on the type of support being used.

By "EST" or "Expressed Sequence Tag" is meant a partial DNA or cDNA sequence of about 150 to 500, more preferably about 300, sequential nucleotides of a longer sequence obtained from a genomic or cDNA library prepared from a selected cell, cell type, tissue or tissue type, organ or organism which longer

sequence corresponds to an mRNA of a gene found in that library. An EST is generally DNA. One or more libraries made from a single tissue type typically provide at least about 3000 different (i.e., unique) ESTs and potentially the full complement of all possible ESTs representing all cDNAs e.g., 50,000-100,000 in an animal such as a human. Further background and information on the construction of ESTs is described in M. D. Adams et al, Science, 252:1651-1656 (1991); and International Application Number PCT/US92/05222 (January 7, 1993).

As used herein, the term "defined oligonucleotide/polynucleotide sequence" refers to a known nucleotide sequence fragment of a selected EST or gene. This term is used interchangeably with the term "fragments of EST". These sequential sequences are generally comprised of between about 15 to about 45 nucleotides and more preferably between about 20 to about 25 nucleotides in length. Thus any single EST of 300 nucleotides in length may provide about 280 different defined oligonucleotide/polynucleotide sequences of 20 nucleotides in length (e.g., 20-mers). The lengths of the defined oligonucleotide/polynucleotides may be readily increased or decreased as desired or needed, depending on the limitations of the solid support on which they may be immobilized or the requirements of the hybridization conditions to be employed. The length is generally guided by the principle that it should be of sufficient length to insure that it is one average only represented once in the population to be examined. Generally, these defined oligonucleotide/polynucleotides are RNA or DNA and are preferably derived from the anti-sense strand of the EST sequence or from a corresponding mRNA sequence to enable their hybridization with samples of RNA or DNA. Modified nucleotides may be incorporated to increase stability and hybridization properties.

By the term "plurality of defined oligonucleotide/polynucleotide sequences" is meant the following. A surface of a solid support may immobilize a large number of "defined oligonucleotide/polynucleotides". For example, depending upon the nature of the surface, it can immobilize from about 300 to upwards of 60,000 defined 20-mer oligonucleotide/polynucleotides. It is anticipated that future improvements to solid surfaces will permit considerably larger such pluralities to be immobilized on a single surface. A "plurality" of sequences refers to the use on any one solid support of multiple different defined oligonucleotide/polynucleotides from a single EST from a selected library, as well as multiple different defined oligonucleotide/polynucleotides from different ESTs from the same library or many libraries from the same or different tissues, and may also include multiple identical copies of defined oligonucleotide/polynucleotides. Ultimately a plurality has at least one oligonucleotide/polynucleotide per expressed gene in the entire organism. For example, from a library producing about 5,000-10,000 ESTs, a single support can

include at least about 1-20 defined oligonucleotide/polynucleotides representing every EST in that library. The composition of defined oligonucleotide/polynucleotides which make up a surface according to this invention may be selected or designed as desired.

5 The term "sample" is employed in the description of this invention in several important ways. As used herein, the term "sample" encompasses any cell or tissue from an organism. Any desired cell or tissue type in any desired state may be selected to form a sample. For example, the sample cell desired may be a human T cell; the desired cell type for use in this invention may be a quiescent T cell or an
10 activated T cell.

By the phrase "analogous sample" or "analogous cell or tissue" is meant that according to this invention when the ESTs which provide the defined oligonucleotide/polynucleotides are produced from a cDNA library prepared from a single tissue or cell type source sample, e.g., liver tissue of a human, then the samples
15 used to hybridize to those immobilized defined oligonucleotide/polynucleotides are preferably provided by the same type of sample from either a healthy or diseased animal, i.e., liver tissue of a healthy human and liver tissue of a diseased or infected human or from a human suspected of having that disease or infection. Alternatively, if the surface contains defined oligonucleotide/polynucleotides from multiple cells or
20 tissues, then the "samples" which are hybridized thereto can be but are not limited to samples obtained from analogous multiple tissues or cells.

By the term "detectably hybridizing" means that the sample from the healthy organism or diseased or infected organism is contacted with the defined oligonucleotide/polynucleotides on the surface for sufficient time to permit the
25 formation of patterns of hybridization on the surfaces caused by hybridization between certain polynucleotide sequences in the samples with the certain immobilized defined oligonucleotide/polynucleotides. These patterns are made detectable by the use of available conventional techniques, such as fluorescent labelling of the samples. Preferably hybridization takes place under stringent conditions, e.g., revealing
30 homologies of about 95%. However, if desired, other less stringent conditions may be selected. Techniques and conditions for hybridization at selected stringencies are well known in the art [see, e.g., Sambrook et al, Molecular Cloning. A Laboratory Manual., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1989)].

35 II. Compositions of The Invention

The present invention is based upon the use of ESTs from any desired cell or tissue in known technologies for oligonucleotide/polynucleotide hybridization.

A. ESTs

An EST, as defined above, is for an animal, a sequence from a cDNA clone that corresponds to an mRNA. The EST sequences useful in the present invention are isolated preferably from cDNA libraries using a rapid screening and sequencing technique. Custom made cDNA libraries are made using known techniques. See, generally, Sambrook et al, cited above. Briefly, mRNA from a selected cell or tissue is reverse transcribed into complementary DNA (cDNA) using the reverse transcriptase enzyme and made double-stranded using RNase H coupled with DNA polymerase or reverse transcriptase. Restriction enzyme sites are added to the cDNA and it is cloned into a vector. The result is a cDNA library. Alternatively, commercially available cDNA libraries may be used. Libraries of cDNA can also be generated from recombinant expression of genomic DNA using known techniques, including polymerase chain reaction-derived techniques.

ESTs (which can range from about 150 to about 500 nucleotides in length, preferably about 300 nucleotides) can be obtained through sequence analysis from either end of the cDNA insert. Desirably, the DNA libraries used to obtain ESTs use directional cloning methods so that either the 5' end of the cDNA (likely to contain coding sequence) or the 3' end (likely to be a non-coding sequence) can be selectively obtained.

In general, the method for obtaining ESTs comprises applying conventional automated DNA sequencing technology to screen clones, advantageously randomly selected clones, from a cDNA library. The cDNA libraries from the desired tissue can be preprocessed, or edited, by conventional techniques to reduce repeated sequencing of high and intermediate abundance clones and to maximize the chances of finding rare messages from specific cell populations. Preferably, preprocessing includes the use of defined composition prescreening probes, e.g., cDNA corresponding to mitochondria, abundant sequences, ribosomes, actins, myelin basic polypeptides, or any other known high abundance peptide. These prescreening probes used for preprocessing are generally derived from known ESTs. Other useful preprocessing techniques include subtraction hybridization, which preferentially reduces the population of highly represented sequences in the library [e.g., see Fargnoli et al, Anal. Biochem., 187:364 (1990)] and normalization, which results in all sequences being represented in approximately equal proportions in the library [Patanjali et al, Proc. Natl. Acad. Sci. USA, 88:1943 (1991)]. Additional prescreening/differential screening approaches are known to those skilled in the art.

ESTs can then be generated from partial DNA sequencing of the selected clones. The ESTs useful in the present invention are preferably generated using low redundancy of sequencing, typically a single sequencing reaction. While

single sequencing reactions may have an accuracy as low as 90%, this nevertheless provides sufficient fidelity for identification of the sequence and design of PCR primers.

If desired, the location of an EST in a full length cDNA is determined by analyzing the EST for the presence of coding sequence. A conventional computer program is used to predict the extent and orientation of the coding region of a sequence (using all six reading frames). Based on this information, it is possible to infer the presence of start or stop codons within a sequence and whether the sequence is completely coding or completely non-coding or a combination of the two. If start or stop codons are present, then the EST can cover both part of the 5'-untranslated or 3'-untranslated part of the mRNA (respectively) as well as part of the coding sequence. If no coding sequence is present, it is likely that the EST is derived from the 3' untranslated sequence due to its longer length and the fact that most cDNA library construction methods are biased toward the 3' end of the mRNA. It should be understood that both coding and non-coding regions may provide ESTs equally useful in the described invention.

A number of specific ESTs suitable for use in the present invention are described above Adams et al (*supra*), which may be incorporated by reference herein, to describe non-essential examples of desirable ESTs. Other ESTs exist in the art which may also be useful in this invention, as will ESTs yet to be developed by these known techniques.

B. Preparing the Solid Support of the Invention

Oligonucleotide sequences which are fragments of defined sequence are derived from each EST by conventional means, e.g., conventional chemical synthesis or recombinant techniques. Each defined oligonucleotide/polynucleotide sequence as described above is a fragment, can be, but is not necessarily an anti-sense fragment, of an EST isolated from a DNA library prepared from a selected cell or tissue type from a selected animal. For use in the present invention, it is presently preferred that the defined oligonucleotide/polynucleotide sequences are 20-25mers. As described above, for each EST a number of such 20-25mers may be generated. The lengths may vary as described above as well as the composition. For example oligonucleotide/polynucleotides can be modified based on the Oligo 4.0 or similar programs to predict hybridization potential or to include modified nucleotides for the reasons given above. It is also appreciated that large DNA segments may be employed including entire ESTs or even full length genes particular when inserted into cloning vectors.

A plurality of these defined oligonucleotide/polynucleotide sequences are then attached to a selected solid support conventionally used for the attachment of nucleotide sequences again by known means. In contrast to other technologies available in the art, this support is designed to contain defined, not random, oligonucleotide/polynucleotide sequences. The EST fragments, or defined oligonucleotide/polynucleotide sequences, immobilized on the solid support can include fragments of one or more ESTs from a library of at least one selected tissue or cell sample of a healthy animal, at least one analogous sample of the animal having a disease, at least one analogous sample of the animal infected with a pathogen, and any combination thereof.

Numerous conventional methods are employed for attaching biological molecules such as oligonucleotide/polynucleotide sequences to surfaces of a variety of solid supports. See, e.g., Affinity Techniques, Enzyme Purification: Part B, Methods in Enzymology, Vol. 34, ed. W.B. Jakoby, M. Wilcheck, Acad. Press, NY (1974); Immobilized Biochemicals and Affinity Chromatography, Advances in Experimental Medicine and Biology, vol. 42, ed. R. Dunlap, Plenum Press, NY (1974); U. S. Patent No. 4,762,881; U. S. Patent No. 4,542,102; European Patent Publication No. 391,608 (October 10, 1990); U. S. Patent No. 4,992,127 (Nov. 21, 1989).

One desirable method for attaching oligonucleotide/polynucleotide sequences derived from ESTs to a solid support is described in International Application No. PCT/US90/06607 (published May 30, 1991). Briefly, this method involves forming predefined regions on a surface of a solid support, where the predefined regions are capable of immobilizing ESTs. The methods make use of binding substances attached to the surface which enable selective activation of the predefined regions. Upon activation, these binding substances become capable of binding and immobilizing oligonucleotide/polynucleotides based on EST or longer gene sequences.

Any of the known solid substrates suitable for binding oligonucleotide/polynucleotides at pre-defined regions on the surface thereof for hybridization and methods for attaching the oligonucleotide/polynucleotides thereto may be employed by one of skill in the art according to this invention. Similarly, known conventional methods for making hybridization of the immobilized oligonucleotide/polynucleotides detectable, e.g., fluorescence, radioactivity, photoactivation, biotinylation, solid state circuitry, and the like may be used in this invention.

Thus, by resorting to known techniques, the invention provides a composition suitable for use in hybridization which consists of a surface of a solid

support on which is immobilized at pre-defined regions on said surface a plurality of defined oligonucleotide/polynucleotide sequences for hybridization. For example, one composition of this invention is a solid support on which are immobilized oligos of EST fragments from a library constructed from a single cell type, e.g., a human stem cell, or a single tissue, e.g., human liver, from a healthy human. Still another composition of this invention is another solid support on which are immobilized oligos of EST fragments from a library constructed from a single cell type or a tissue from a human having a selected disease or predisposition to a selected disease, e.g., liver cancer.

Another embodiment of the compositions of this invention include a single solid support having oligonucleotides of ESTs from both single cell or single tissue libraries from both a healthy and diseased human. Still other embodiments include a single support on which are immobilized oligos of EST fragments from more than one tissue or cell library from a healthy human or a single support on which are immobilized more than one tissue or cell library from both healthy and diseased animals or humans. A preferred composition of this invention is anticipated to be a single support containing oligos of ESTs for all known cells and tissues from a selected organism.

III. *The Methods of the Invention*

A. *Identification of Genes*

The present invention employs the compositions described above in methods for identifying genes which are differentially expressed in a normal healthy organism and an organism having a disease or infection. These methods may be employed to detect such genes, regardless of the state of knowledge about the function of the gene. The method of this invention by use of the compositions containing multiple defined EST fragments from a single gene as described above is able to detect levels of expression of genes or in other cases simply the expression or lack thereof, which differ between normal, healthy organisms and organisms having a selected disease, disorder or infection.

One such method employs a first surface of a solid support on which is immobilized at pre-defined regions thereon a plurality of defined oligonucleotide/polynucleotide sequences, described above, of EST or longer gene fragment isolated from a cDNA library prepared from at least one selected tissue or cell sample of a healthy animal (the "healthy test surface") and a second such surface on which is immobilized at pre-defined regions a plurality of defined oligonucleotide/polynucleotide sequences of EST or longer gene fragment isolated from at least one analogous tissue of an animal having a selected disease (the "disease

test surface"). These test surfaces may be standardized for the selected animal or selected cell or tissue sample from that animal (i.e., they are prescreened for polymorphisms in the species population).

Polynucleotide sequences are then isolated from mRNA and/or
5 cDNA from a biological sample from a known healthy animal ("healthy control") and a second sample is similarly prepared from a sample from a known diseased animal ("disease sample"). These two samples are desirably selected from the cell or tissue analogous to that which provided the immobilized oligonucleotide/polynucleotides.

According to the method the healthy control sample is
10 contacted with one set of the healthy test surface and the disease test surface described above for a time sufficient to permit detectable hybridization to occur between the sample and the immobilized defined oligonucleotide/polynucleotides on each surface. The results of this hybridization are a first hybridization pattern formed between the nucleotides of healthy control and the healthy test surface and a second
15 hybridization pattern formed between the nucleotides of healthy control sample and the disease test surface.

In a similar manner, the disease sample is detectably hybridized to another set of healthy test and disease test surfaces, forming a third hybridization pattern between the disease sample and healthy test surface and a fourth hybridization
20 pattern between the disease sample and the disease test surface.

Comparing the four hybridization patterns permits detection of those defined oligonucleotide/polynucleotides which are differentially expressed between the healthy control and the disease sample by the presence of differences in the hybridization patterns at pre-defined regions. The
25 oligonucleotide/polynucleotides on each surface which correspond to the pattern differences may be readily identified with the corresponding EST or longer gene fragment from which the oligonucleotide/polynucleotides are obtained.

In another embodiment of the method of this invention, the same process is employed, with the exception that plurality of defined
30 oligonucleotide/polynucleotide sequences forming the healthy test sample and the disease test sample surfaces are immobilized on a single solid support. For example, each fragment of an EST or longer gene fragment on the surface is isolated from at least two cDNA libraries prepared from a selected cell or tissue sample of a healthy animal and an analogous selected cell or tissue sample of an animal having a disease.

35 According to this embodiment, the healthy control sample is detectably hybridized to a copy of this single solid surface, forming one hybridization pattern with oligonucleotide/polynucleotides associated with both the healthy and diseased animal. Similarly, the disease sample is detectably hybridized to a second

copy of this single solid surface, forming one hybridization pattern with oligonucleotide/polynucleotides associated with both the healthy and diseased animal.

5 Comparing the two hybridization patterns permits detection of those defined oligonucleotide/polynucleotides which are differentially expressed between the healthy control and the disease sample by the presence of differences in the hybridization patterns at pre-defined regions. The oligonucleotide/polynucleotides on each surface which correspond to the pattern differences may be readily identified with the corresponding EST or longer gene fragment from which the oligonucleotide/polynucleotides are obtained.

10 The identification of one or more ESTs as the source of the defined oligonucleotide/polynucleotide which produced a "difference" in hybridization patterns according to these methods permits ready identification of the gene from which those ESTs were derived. Because oligonucleotides are of sufficient length that they will hybridize under stringent conditions only with a RNA/cDNA for
15 that gene to which they correspond, the oligo can be used to identify the EST and in turn the clone from which it was derived and by subsequent cloning, obtain the sequence of the full-length cDNA and its genomic counterparts, i.e., the gene, from which it was obtained.

In other words, the ESTs identified by the method of this
20 invention can be employed to determine the complete sequence of the mRNA, in the form of transcribed cDNA, by using the EST as a probe to identify a cDNA clone corresponding to a full-length transcript, followed by sequencing of that clone. The EST or the full length cDNA clone can also be used as a probe to identify a genomic clone or clones that contain the complete gene including regulatory and promoter
25 regions, exons, and introns.

It should be appreciated that one does not have to be restricted in using ESTs from a particular tissue from which probe RNA or cDNA is obtained, rather any or all ESTs (known or unknown) may be placed on the support. Hybridization will be used to form diagnostic patterns or to identify which particular
30 EST is detected. For example, all known ESTs from an organism are used to produce a "master" solid support to which control sample and disease samples are alternately hybridized. One then detects a pattern of hybridization associated with the particular disease state which then forms the basis of a diagnostic test or the isolation of disease specific ESTs from which the intact gene may be cloned and sequenced
35 leading ultimately to a defined therapeutic target.

Methods for obtaining complete gene sequences from ESTs are well-known to those of skill in the art. See, generally, Sambrook et al, cited above. Briefly, one suitable method involves purifying the DNA from the clone that was

sequenced to give the EST and labeling the isolated insert DNA. Suitable labeling systems are well known to those of skill in the art [see, eg. Basic Methods in Molecular Biology, L. G. Davis et al, ed., Elsevier Press, NY (1986)]. The labeled EST insert is then used as a probe to screen a lambda phage cDNA library or a plasmid cDNA library, identifying colonies containing clones related to the probe cDNA which can be purified by known methods. The ends of the newly purified clones are then sequenced to identify full length sequences and complete sequencing of full length clones is performed by enzymatic digestion or primer walking. A similar screening and clone selection approach can be applied to clones from a genomic DNA library.

Additionally, an EST or gene identified by this method as associated with inherited disorders can be used to determine at what stage during embryonic development the selected gene from which it is derived is developed by screening embryonic DNA libraries from various stages of development, e.g. 2-cell, 8-cell, etc., for the selected gene. As has been mentioned above, the invention may be applied in additional temporal modes for monitoring the progression of a disease state, the efficacy of a particular treatment modality or the aging process of an individual.

Thus, the methods of this invention permit the identification, isolation and sequencing of a gene which is differentially expressed in a selected disease/infection. As described in more detail below, the identified gene may then be employed to obtain any protein encoded thereby, or may be employed as a target for diagnostic methods or therapeutic approaches to the treatment of the disease, including, e.g., drug development.

The same methods as described above for the identification of genes, including genes of unknown function, which are differentially expressed in a disease state, may also be employed to identify other genes of interest. For example, another embodiment of this invention includes a method for identifying a gene of a pathogen which is expressed in a biological sample of an animal infected with that pathogen or the gene of the host which is altered in its expression as a result of the infection.

One such method employs a healthy test surface as described above, employing defined oligonucleotide/polynucleotides from a sample of a healthy, uninfected animal. The second such surface has immobilized at pre-defined regions thereon a plurality of defined oligonucleotide/polynucleotide sequences of ESTs isolated from at least one analogous tissue or cell sample of an infected animal (the "infection test surface"). Polynucleotide sequences are isolated from a biological sample from a healthy animal ("healthy control") and a second sample is similarly

prepared from an animal infected with the selected pathogen ("infection sample"). These two samples are desirably selected from the cell or tissue analogous to that which provided the immobilized oligonucleotide/polynucleotides. It would also be possible to provide samples from the nucleic acid of the pathogen itself.

5 According to the method the healthy control sample is contacted with one set of the healthy test surface and the infection test surface described above for a time sufficient to permit detectable hybridization to occur between the sample and the immobilized defined oligonucleotide/polynucleotides on each surface. The results of this hybridization are a first hybridization pattern formed
10 between the nucleotides of healthy control and the healthy test surface and a second hybridization pattern formed between the nucleotides of healthy control sample and the infection test surface.

 In a similar manner, the infection sample is detectably hybridized to another set of healthy test and infection test surfaces, forming a third
15 hybridization pattern between the infection sample and healthy test surface and a fourth hybridization pattern between the infection sample and the infection test surface.

 Comparing the four hybridization patterns permits detection of those defined oligonucleotide/polynucleotides which are differentially expressed
20 between the healthy animal and the animal infected with the pathogen by the presence of differences in the hybridization patterns at pre-defined regions. As mentioned differential expression is not required and simple qualitative analysis is possible by reference to gene expression which is simply present or absent.

 A second embodiment of this method parallels the second
25 embodiment of the method as applied to disease above, i.e., the same process is employed, with the exception that plurality of defined oligonucleotide/polynucleotide sequences forming the healthy test sample surface and the infection test sample surface are immobilized on a single solid support. The resulting first hybridization pattern (healthy control sample with healthy/infection test sample) and second
30 hybridization pattern (infection sample with healthy/infection test sample) permits detection of those defined oligonucleotide/polynucleotides which are differentially expressed between the healthy control and the infection sample by the presence of differences in the hybridization patterns at pre-defined regions. The oligonucleotide/polynucleotides on each surface which correspond to the pattern
35 differences may be readily identified with the corresponding ESTs from which the oligonucleotide/polynucleotides are obtained.

 As described above for the methods for identifying differential gene expression between diseased and healthy animals, the

oligonucleotide/polynucleotides on each surface which correspond to the pattern differences may be readily identified with the corresponding ESTs from which the oligonucleotide/polynucleotide sequences are obtained and the genes expressed by the pathogen identified for similar purposes. Other embodiments of these methods may be developed with resort to the teaching herein, by altering the samples which provide the defined oligonucleotide/polynucleotides. For example, an EST, identified with a differentially expressed gene by the method of this invention is also useful in detecting genes expressed in the various stages of an pathogen's development, particularly the infective stage and following the cours of drug treatment and emergence of resistant variants. For example, employing the techniques described above, the EST can be used for detecting a gene in various stages of the parasitic *Plasmodium* species life cycle, which include blood stages, liver stages, and gametocyte stages.

B. Diagnostic Methods

In addition to use of the methods and compositions of this invention for identifying differentially expressed genes, another embodiment of this invention provides diagnostic methods for diagnosing a selected disease state, or a selected state resulting from aging, exposure to drugs or infection in an animal. According to this aspect of the invention, a first surface, described as the healthy test surface above, and a second surface, described as the disease test surface or infection test surface, are prepared depending on the disease or infection to be diagnosed. The same processes of detectable hybridization to a first and second set of these surfaces with the healthy control sample and disease/infection sample are followed to provide the four above-described hybridization patterns, i.e., healthy control sample with healthy test surface; healthy control sample with disease/infection test surface; disease/infection sample with healthy test surface; and disease/infection sample with disease/infection test surface.

The diagnosis of disease or infection is provided by comparing the four hybridization patterns. Substantial differences between the first and third hybridization patterns, respectively, and the second and fourth hybridization patterns, respectively, indicate the presence of the selected disease or infection in said animal. Substantial similarities in the first and third hybridization patterns and second and fourth hybridization patterns indicates the absence of disease or infection.

A similar embodiment utilizes the single surface bearing both the healthy test surface defined oligonucleotide/polynucleotides and the disease/infection test surface defined oligonucleotide/polynucleotides as described above. Parallel process steps as described above for detection of genes differentially expressed in disease and infected states are followed, resulting in a first hybridization

pattern (healthy control sample with single healthy and disease/infection test sample) and a second hybridization pattern (disease/infection sample with another copy of the single healthy and disease/infection test sample).

5 Diagnosis is accomplished by comparing the two hybridization patterns, wherein substantial differences between the first and second hybridization patterns indicate the presence of the selected disease or infection in the animal being tested. Substantially similar first and second hybridization patterns indicate the absence of disease or infection. This like many of the foregoing embodiments may use known or unknown ESTs derived from many libraries.

10 C. *Other Methods of the Invention*

As is obvious to one of skill in the art upon reading this disclosure, the compositions and methods of this invention may also be used for other similar purposes. For example, the general methods and compositions may be adapted easily by manipulation of the samples selected to provide the standardized
15 defined oligonucleotide/polynucleotides, and selection of the samples selected for hybridization thereto. One such modification is the use of this invention to identify cell markers of any type, e.g., markers of cancer cells, stem cell markers, and the like. Another modification involves the use of the method and compositions to generate hybridization patterns useful for forensic identification or an 'expression fingerprint'
20 of genes for identification of one member of a species from another. Similarly, the methods of this invention may be adapted for use in tissue matching for transplantation purposes as well as for molecular histology, i.e., to enable diagnosis of disease or disorders in pathology tissue samples such as biopsies. Still another use of this method is in monitoring the effects of development and aging upon the gene
25 expression in a selected animal, by preparing surfaces bearing oligonucleotide/polynucleotides prepared from samples of standardized younger members of the species being tested. Additionally the patient can serve as an internal control by virtue of having the method applied to blood samples every 5-10 years during his lifetime.

30 Still another intriguing use of this method is in the area of monitoring the effects of drugs on gene expression, both in laboratories and during clinical trials with animal, especially humans. Because the method can be readily adapted by altering the above parameters, it can essentially be employed to identify differentially expressed genes of any organism, at any stage of development, and
35 under the influence of any factor which can affect gene expression.

IV. *The Genes and Proteins Identified*

Application of the compositions and methods of this invention as above described also provide other compositions, such as any isolated gene sequence which is differentially expressed between a normal healthy animal and an animal having a disease or infection. Another embodiment of this invention is any isolated pathogen gene sequence which is expressed in tissue or cell samples of an infected animal. Similarly an embodiment of this invention is any gene sequence identified by the methods described herein.

These gene sequences may be employed in conventional methods to produce isolated proteins encoded thereby. To produce a protein of this invention, the DNA sequences of a desired gene identified by the use of the methods of this invention or portions thereof are inserted into a suitable expression system. Desirably, a recombinant molecule or vector is constructed in which the polynucleotide sequence encoding the protein is operably linked to a heterologous expression control sequence permitting expression of the human protein. Numerous types of appropriate expression vectors and host cell systems are known in the art for mammalian (including human) expression, insect, e.g., baculovirus expression, yeast, fungal, and bacterial expression, by standard molecular biology techniques.

The transfection of these vectors into appropriate host cells, whether mammalian, bacterial, fungal, or insect, or into appropriate viruses, can result in expression of the selected proteins. Suitable host cells or cell lines for transfection, and viruses, as well as methods for the construction and transfection of such host cells and viruses are well-known. Suitable methods for transfection, culture, amplification, screening, and product production and purification are also known in the art.

The genes and proteins identified by this invention can be employed, if desired in diagnostic compositions useful for the diagnosis of a disease or infection using conventional diagnostic assays. For example, a diagnostic reagent can be developed which detectably targets a gene sequence or protein of this invention in a biological sample of an animal. Such a reagent may be a complementary nucleotide sequence, an antibody (monoclonal, recombinant or polyclonal), or a chemically derived agonist or antagonist. Alternatively, the proteins and polynucleotide sequences of this invention, fragments of same, or complementary sequences thereto, may themselves be useful as diagnostic reagents for diagnosing disease states with which the ESTs of the invention are associated. These reagents may optionally be labelled using diagnostic labels, such as radioactive labels, colorimetric enzyme label systems and the like conventionally used in diagnostic or therapeutic methods, e.g., Northern and Western blotting, antigen-antibody binding and the like. The selection of the appropriate assay format and label system is within the skill of the art and may

readily be chosen without requiring additional explanation by resort to the wealth of art in the diagnostic area.

Additionally, genes and proteins identified according to this invention may be used therapeutically. For example, the EST-containing gene sequences may be useful in gene therapy, to provide a gene sequence which in a disease is not properly or sufficiently expressed. In such a method, a selected gene sequence of this invention is introduced into a suitable vector or other delivery system for delivery to a cell containing a defect in the selected gene. Suitable delivery systems are well known to those of skill in the art and enable the desired EST or gene to be incorporated into the target cell and to be translated by the cell. The EST or gene sequence may be introduced to mutate the existing gene by recombination or provide an active copy thereof in addition to the inactive gene to replace its function.

Alternatively, a protein encoded by an EST or gene of the invention may be useful as a therapeutic reagent for delivery of a biologically active protein, particularly when the disease state is associated with a deficiency of this protein. Such a protein may be incorporated into an appropriate therapeutic formulation, alone or in combination with other active ingredients. Methods of formulating such therapeutic compositions, as well as suitable pharmaceutical carriers, and the like, are well known to those of skill in the art. Still an additional method of delivering the missing protein encoded by an EST, or the gene from which a selected EST was derived, involves expressing it directly *in vivo*. Systems for such *in vivo* expression are well known in the art.

Yet another use of the ESTs, genes identified according to the methods of this invention, or the proteins encoded thereby is a target for the screening and development of natural or synthetic chemical compounds which have utility as therapeutic drugs for the treatment of disease states associated with the identified genes and ESTs derived therefrom. As one example, a compound capable of binding to such a protein encoded by such a gene and either preventing or enhancing its biological activity may be a useful drug component for the treatment or prevention of such disease states.

Conventional assays and techniques may be used for the screening and development of such drugs. As one example, a method for identifying compounds which specifically bind to or inhibit or activate proteins encoded by these gene sequences can include simply the steps of contacting a selected protein or gene product, with a test compound to permit binding of the test compound to the protein; and determining the amount of test compound, if any, which is bound to the protein. Such a method may involve the incubation of the test compound and the protein immobilized on a solid support. Still other conventional methods of drug screening

can involve employing a suitable computer program to determine compounds having similar or complementary chemical structures to that of the gene product or portions thereof and screening those compounds either for competitive binding to the protein to detect enhanced or decreased activity in the presence of the selected compound.

5 Thus, through use of such methods, the present invention is anticipated to provide compounds capable of interacting with these genes, ESTs, or encoded proteins, or fragments thereof, and either enhancing or decreasing the biological activity, as desired. Such compounds are believed to be encompassed by this invention.

10 Numerous modifications and variations of the present invention are included in the above-identified specification and are expected to be obvious to one of skill in the art. Such modifications and alterations to the compositions and processes of the present invention are believed to be encompassed in the scope of the claims appended hereto.

15

WHAT IS CLAIMED IS:

1. A method for identifying genes which are differentially expressed in two different pre-determined states of an organism comprising:
 - 5 a. providing a first surface on which is immobilized at pre-defined regions on said surface a plurality of defined oligonucleotide/polynucleotide sequences, each sequence selected from the group consisting of a fragment of an EST, an entire EST a fragment of a gene or an entire gene, isolated from a DNA library prepared from at least one selected cell, tissue, organ or organism sample in a first
10 state and present in excess relative to the polynucleotide to be hybridized;
 - b. providing a second surface on which is immobilized at pre-defined regions on said surface a plurality of defined oligonucleotide/polynucleotide sequences, each sequence selected from the group consisting of a fragment of an EST, an entire EST a fragment of a gene or an entire gene, isolated from a DNA library
15 prepared from at least one selected cell, tissue, organ or organism sample in a second state and present in excess relative to the polynucleotide to be hybridized;
 - c. detectably hybridizing to a set of said first and second surfaces polynucleotide sequences isolated from a sample from a said organism in said first state, said sample selected from sources analogous to the sources of step (a), said
20 hybridization sufficient to form a first and second hybridization pattern on each said first and second surface,
 - d. detectably hybridizing to a set of said first and second surfaces polynucleotide sequences isolated from a sample from said organism in said second state, said sample selected from sources analogous to the sources of step (c), said
25 hybridization sufficient to form a third and fourth hybridization pattern on each said first and second surface,
 - e. comparing at least two of the four hybridization patterns, wherein genes differentially expressed in said first and second states are identified by the presence of differences in the hybridization patterns at pre-defined regions;
 - 30 f. identifying the oligonucleotide/polynucleotides on each surface which correspond to said pattern differences and the corresponding ESTs or larger gene fragment from which the oligonucleotide/polynucleotides were obtained, whereby identification of the EST or larger gene fragment permits identification of the gene from which the ESTs or larger gene fragment were derived.

35

2. The method according to Claim 1 wherein said first and second states are respectively healthy and disease; pathogen uninfected and pathogen infected; a first progression state and a second progression of a disease or infection; a first treatment state and a second treatment state of a disease or infection; or a first developmental and a second developmental state.

3. The method according to Claim 1 wherein said organism is a plant or an animal.

4. The method according to Claim 3 wherein said animal is a human.

5. A method for identifying genes which are differentially expressed in a normal healthy animal and an animal having a disease comprising:

a. providing a first surface on which is immobilized at pre-defined regions on said surface a plurality of defined oligonucleotide/polynucleotide sequences, each sequence each sequence selected from the group consisting of a fragment of an EST, an entire EST a fragment of a gene or an entire gene, isolated from a DNA library prepared from at least one selected cell, tissue, organ or organism sample in a healthy animal and present in excess relative to the polynucleotide to be hybridized;

b. providing a second surface on which is immobilized at pre-defined regions of said surface a plurality of defined oligonucleotide/polynucleotide sequences, each sequence each sequence selected from the group consisting of a fragment of an EST, an entire EST a fragment of a gene or an entire gene, isolated from a DNA library prepared from at least one selected cell, tissue, organ or organism sample from an animal having said disease and present in excess relative to the polynucleotide to be hybridized;

c. detectably hybridizing to a set of said first and second surfaces polynucleotide sequences isolated from a sample from a healthy animal, said sample selected from sources analogous to the sources of step (a), said hybridization sufficient to form a first and second hybridization pattern on each said first and second surface, said sample selected from a cell or tissue sample analogous to the sample of step (a), said hybridization sufficient to form a first and second hybridization pattern on each said first and second surface;

- 5 d. detectably hybridizing to a set of said first and second surfaces polynucleotide sequences isolated from a sample from an animal having said disease, said sample selected from a cell or tissue sample analogous to the sample of step (c), said hybridization sufficient to form a third and fourth hybridization pattern on each said first and second surface,
- e. comparing at least two of the four hybridization patterns, wherein genes differentially expressed in said first and second states are identified by the presence of differences in the hybridization patterns at pre-defined regions;
- 10 f. identifying the oligonucleotide/polynucleotides on each surface which correspond to said pattern differences and the corresponding ESTs or larger gene fragment from which the oligonucleotide/polynucleotides were obtained, whereby identification of the EST or larger gene fragment permits identification of the gene from which the ESTs or larger gene fragment were derived.
- 15 6. A method for identifying genes which are differentially expressed in a normal healthy animal and an animal having a disease comprising:
- a. providing a surface on which is immobilized at pre-defined regions on said surface a plurality of defined oligonucleotide/polynucleotide sequences, each sequence selected from the group consisting of a fragment of an EST, an entire EST a fragment of a gene or an entire gene isolated from a DNA library prepared from the group selected from at least one selected cell, tissue, organ or organism sample in of a healthy animal and an analogous selected sample of an animal having said disease and both present in excess relative to the polynucleotide to be hybridized;
- 20 b. detectably hybridizing to a first copy of said surface polynucleotide sequences isolated from a healthy animal, said sample selected from a cell or tissue sample analogous to the sample of step (a), said hybridization sufficient to form a first hybridization pattern on said surface;
- c. detectably hybridizing to a second copy of said surface polynucleotide sequences isolated from an animal having said disease, said sample selected from a cell or tissue sample analogous to the sample of step (a), said hybridization sufficient to form a second hybridization pattern on said surface;
- 30 d. comparing the two hybridization patterns, wherein genes differentially expressed in a disease state are identified by the presence of differences in the hybridization patterns at pre-defined regions;
- 35

e. identifying the oligonucleotide/polynucleotides on each surface which correspond to said pattern differences and the corresponding ESTs from which the oligonucleotide/polynucleotides are obtained, whereby identification of the EST permits identification of the gene from which the ESTs were derived.

5

7. A method for identifying a gene of a pathogen which is expressed in a biological sample of an animal infected with said pathogen comprising:

a. providing a first surface on which is immobilized at pre-defined regions on said surface a plurality of defined oligonucleotide/polynucleotide sequences, each sequence selected from the group consisting of a fragment of an EST, an entire EST a fragment of a gene or an entire gene isolated from a DNA library prepared from at least one selected cell, tissue, organ or organism sample of a healthy, uninfected animal and present in excess relative to the polynucleotide to be hybridized;

15

b. providing a second surface on which is immobilized at pre-defined regions of said surface a plurality of defined oligonucleotide/polynucleotide sequences, each sequence selected from the group consisting of a fragment of an EST, an entire EST a fragment of a gene or an entire gene isolated from at least one selected cell, tissue, organ or organism sample of an infected animal;

20

c. detectably hybridizing to a set of said first and second surfaces polynucleotide sequences isolated from a sample from a healthy animal, said sample selected from a cell or tissue sample analogous to the sample of step (a), said hybridization sufficient to form first and second hybridization patterns on each said first and second surface,

25

d. detectably hybridizing to a set of said first and second surfaces polynucleotide sequences isolated from a sample from an infected animal, said sample selected from a cell or tissue sample analogous to the sample of step (a), said hybridization sufficient to form third and fourth hybridization patterns on each said first and second surface,

30

e. comparing the four hybridization patterns, wherein genes of said pathogen which are expressed in an infected animal are identified by the presence of differences in the hybridization patterns at pre-defined regions;

f. identifying the oligonucleotide/polynucleotides on each surface which correspond to said pattern differences and the corresponding ESTs from which the oligonucleotide/polynucleotides are obtained, whereby identification of the EST permits identification of the gene from which the ESTs were derived.

35

8. A method for identifying a gene of a pathogen which is expressed in a biological sample of an animal infected with said pathogen comprising:

- a. providing a surface on which is immobilized at pre-defined regions on said surface a plurality of defined oligonucleotide/polynucleotide sequences, each sequence selected from the group consisting of a fragment of an EST, an entire EST a fragment of a gene or an entire gene isolated from a DNA library prepared from the group selected from at least one selected cell, tissue, organ or organism sample in of a healthy animal and an analogous selected sample of an animal having said disease and both present in excess relative to the polynucleotide to be hybridized
- b. detectably hybridizing to a first copy of said surface polynucleotide sequences isolated from a sample from a healthy animal, said sample selected from a cell or tissue sample analogous to the sample of step (a), said hybridization sufficient to form a first hybridization pattern on said surface;
- c. detectably hybridizing to a second copy of said surface polynucleotide sequences isolated from a sample from an infected animal, said sample selected from a cell or tissue sample analogous to the sample of step (a), said hybridization sufficient to form a second hybridization pattern on said surface;
- d. comparing the two hybridization patterns, wherein genes of said pathogen which are expressed in an infected animal are identified by the presence of differences in the hybridization patterns at pre-defined regions;
- e. identifying the oligonucleotide/polynucleotides on each surface which correspond to said pattern differences and the corresponding ESTs from which the oligonucleotide/polynucleotides are obtained, whereby identification of the EST permits identification of the gene from which the ESTs were derived.

9. A composition suitable for use in hybridization comprising a solid surface on which is immobilized at pre-defined regions on said surface a plurality of defined oligonucleotide/polynucleotide sequences for hybridization, each sequence selected from the group consisting of a fragment of an EST, an entire EST a fragment of a gene or an entire gene isolated from a DNA library prepared from the group selected from at least one selected cell, tissue, organ or organism sample of a healthy animal, at least one analogous sample of said animal having a disease, at least one analogous sample of said animal infected with a microbial pathogen, and any combination thereof.

10. An isolated gene sequence which is differentially expressed in a normal healthy animal and an animal having a disease, identified by the method of claim 1.

5 11. An isolated pathogen gene sequence which is expressed in tissue or cell samples of an infected animal identified by the method of claim 7.

12. A diagnostic composition useful for the diagnosis of a disease comprising a reagent capable of detectably targeting a gene sequence of claim 10 in a
10 biological sample of an animal.

13. A diagnostic composition useful for the diagnosis of infection by a pathogen comprising a reagent capable of detectably targeting a gene sequence of claim 11 in a biological sample of an animal.

15 14. An isolated protein produced by expression of a gene sequence of claim 10.

15. An isolated pathogen protein produced by expression of a gene
20 sequence of claim 11.

16. A therapeutic composition comprising a protein or fragment thereof selected from the group consisting of a protein of claim 10 and a protein of claim 15.

25 17. A method for diagnosing a selected disease or infection in an animal comprising:

a. providing a first surface on which is immobilized at pre-defined regions on said surface a plurality of defined oligonucleotide/polynucleotide sequences, each sequence selected from the group consisting of a fragment of an EST,
30 an entire EST a fragment of a gene or an entire gene, isolated from a DNA library prepared from at least one selected cell, tissue, organ or organism sample of a healthy animal and present in excess relative to the polynucleotide to be hybridized;

b. providing a second surface on which is immobilized at pre-defined regions of said surface a plurality of defined oligonucleotide/polynucleotide
35 sequences, each sequence comprising a fragment of an EST isolated from at least one said tissue of an animal having said disease;

- 5 c. detectably hybridizing to a set of said first and second surfaces polynucleotide sequences isolated from a DNA library prepared from a sample from a healthy animal, said sample selected from a cell or tissue sample analogous to the sample of step (a), said hybridization sufficient to form a first and second hybridization pattern on each said first and second surface;
- 10 d. detectably hybridizing to a set of said first and second surfaces polynucleotide sequences isolated from a DNA library prepared from a sample from an animal having said disease, said sample selected from a cell or tissue sample analogous to the sample of step (c), said hybridization sufficient to form a third and fourth hybridization pattern on each said first and second surface;
- 15 e. comparing the four hybridization patterns, wherein substantial differences between the first and third hybridization patterns and the second and fourth hybridization patterns indicates the presence of said selected disease or infection in said animal, and substantial similarities in said first and third hybridization patterns and second and fourth hybridization patterns indicates the absence of disease or infection.

18. A method for diagnosing a selected disease or infection in an animal comprising:

- 20 a. providing a surface on which is immobilized at pre-defined regions on said surface a plurality of defined oligonucleotide/polynucleotide sequences, each sequence comprising a fragment of an EST isolated from a DNA library prepared from the group consisting of a selected cell or tissue sample of a healthy animal and an analogous selected cell or tissue sample of an animal having
- 25 said disease;
- b. detectably hybridizing to a first copy of said surface polynucleotide sequences isolated from a sample from a healthy animal, said sample selected from a cell or tissue sample analogous to the sample of step (a), said hybridization sufficient to form a first hybridization pattern on said surface;
- 30 c. detectably hybridizing to a second copy of said surface polynucleotide sequences isolated from a DNA library prepared from a sample from an animal having said disease, said sample selected from a cell or tissue sample analogous to the sample of step (a), said hybridization sufficient to form a second hybridization pattern on said surface;
- 35 d. comparing the two hybridization patterns, wherein substantial differences between the first and second hybridization patterns indicates the presence of said selected disease or infection in said animal, and substantial similarities in said first and second hybridization patterns indicates the absence of disease or infection.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US95/01863

A. CLASSIFICATION OF SUBJECT MATTER

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According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

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Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, CAS, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	ANALYTICAL BIOCHEMISTRY, VOLUME 187, ISSUED 1990, FARGNOLI ET AL, "LOW-RATIO HYBRIDIZATION SUBTRACTION", PAGES 364-373, SEE ENTIRE DOCUMENT.	1-18
Y	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES USA, VOLUME 88, ISSUED MARCH 1991, PATANJALI ET AL, "CONSTRUCTION OF A UNIFORM-ABUNDANCE (NORMALIZED) CDNA LIBRARY", PAGES 1943-1947, SEE ENTIRE DOCUMENT.	1-18
Y	SCIENCE, VOLUME 245, ISSUED 29 SEPTEMBER 1989, OLSON ET AL. "A COMMON LANGUAGE FOR PHYSICAL MAPPING OF THE HUMAN GENOME", PAGES 1434-1435, SEE ENTIRE DOCUMENT.	1-18

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	*T	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
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C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	SCIENCE, VOLUME 252, ISSUED 21 JUNE 1991, ADAMS ET AL, "COMPLEMENTARY DNA SEQUENCING: EXPRESSED SEQUENCE TAGS AND HUMAN GENOME PROJECT", PAGES 1651-1656, SEE ENTIRE DOCUMENT.	1-18



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(21) International Application Number: PCT/US95/01160 (22) International Filing Date: 27 January 1995 (27.01.95) (30) Priority Data: 08/187,530 27 January 1994 (27.01.94) US 08/282,955 29 July 1994 (29.07.94) US (71) Applicant: INCYTE PHARMACEUTICALS, INC. [US/US]; 3330 Hillview Avenue, Palo Alto, CA 94304 (US). (72) Inventors: SEILHAMER, Jeffrey, J.; 12555 La Cresta, Los Altos Hills, CA 94022 (US). SCOTT, Randal, W.; 13140 Sun-Mor, Mountain View, CA 94040 (US). (74) Agents: CAGE, Kenneth, L. et al.; Willian Brinks Hofer Gilson & Lione, 2000 K Street, N.W., Suite 200, Washington, DC 20006-1809 (US).		(81) Designated States: AM, AU, BB, BG, BR, BY, CA, CN, CZ, EE, FI, GE, HU, JP, KG, KP, KR, KZ, LK, LR, LT, LV, MD, MG, MN, MX, NO, NZ, PL, RO, RU, SI, SK, TJ, TT, UA, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG), ARIPO patent (KE, MW, SD, SZ). Published <i>With international search report.</i>
(54) Title: COMPARATIVE GENE TRANSCRIPT ANALYSIS (57) Abstract A method and system for quantifying the relative abundance of gene transcripts in a biological specimen. One embodiment of the method generates high-throughput sequence-specific analysis of multiple RNAs or their corresponding cDNAs (gene transcript imaging analysis). Another embodiment of the method produces a gene transcript imaging analysis by the use of high-throughput cDNA sequence analysis. In addition, the gene transcript imaging can be used to detect or diagnose a particular biological state, disease, or condition which is correlated to the relative abundance of gene transcripts in a given cell or population of cells. The invention provides a method for comparing the gene transcript image analysis from two or more different biological specimens in order to distinguish between the two specimens and identify one or more genes which are differentially expressed between the two specimens.		

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COMPARATIVE GENE TRANSCRIPT ANALYSIS

1. FIELD OF INVENTION

The present invention is in the field of molecular biology and computer science; more particularly, the present invention describes methods of analyzing gene transcripts and diagnosing the genetic expression of cells and tissue.

2. BACKGROUND OF THE INVENTION

Until very recently, the history of molecular biology has been written one gene at a time. Scientists have observed the cell's physical changes, isolated mixtures from the cell or its milieu, purified proteins, sequenced proteins and therefrom constructed probes to look for the corresponding gene.

Recently, different nations have set up massive projects to sequence the billions of bases in the human genome. These projects typically begin with dividing the genome into large portions of chromosomes and then determining the sequences of these pieces, which are then analyzed for identity with known proteins or portions thereof, known as motifs. Unfortunately, the majority of genomic DNA does not encode proteins and though it is postulated to have some effect on the cell's ability to make protein, its relevance to medical applications is not understood at this time.

A third methodology involves sequencing only the transcripts encoding the cellular machinery actively involved in making protein, namely the mRNA. The advantage is that the cell has already edited out all the non-coding DNA, and it is relatively easy to identify the protein-coding portion of the RNA. The utility of this approach was not immediately obvious to genomic researchers. In fact, when cDNA sequencing was initially proposed, the method was roundly denounced by those committed to genomic sequencing. For example, the head of the U.S. Human Genome project discounted CDNA sequencing as not valuable and refused to approve funding of projects.

In this disclosure, we teach methods for analyzing DNA, including cDNA libraries. Based on our analyses and

research, we see each individual gene product as a "pixel" of information, which relates to the expression of that, and only that, gene. We teach herein, methods whereby the individual "pixels" of gene expression information can be
5 combined into a single gene transcript "image," in which each of the individual genes can be visualized simultaneously and allowing relationships between the gene pixels to be easily visualized and understood.

We further teach a new method which we call electronic
10 subtraction. Electronic subtraction will enable the gene researcher to turn a single image into a moving picture, one which describes the temporality or dynamics of gene expression, at the level of a cell or a whole tissue. It is that sense of "motion" of cellular machinery on the
15 scale of a cell or organ which constitutes the new invention herein. This constitutes a new view into the process of living cell physiology and one which holds great promise to unveil and discover new therapeutic and diagnostic approaches in medicine.

20 We teach another method which we call "electronic northern," which tracks the expression of a single gene across many types of cells and tissues.

Nucleic acids (DNA and RNA) carry within their sequence the hereditary information and are therefore the
25 prime molecules of life. Nucleic acids are found in all living organisms including bacteria, fungi, viruses, plants and animals. It is of interest to determine the relative abundance of different discrete nucleic acids in different cells, tissues and organisms over time under various
30 conditions, treatments and regimes.

All dividing cells in the human body contain the same set of 23 pairs of chromosomes. It is estimated that these autosomal and sex chromosomes encode approximately 100,000 genes. The differences among different types of cells are
35 believed to reflect the differential expression of the 100,000 or so genes. Fundamental questions of biology could be answered by understanding which genes are transcribed and knowing the relative abundance of transcripts in different cells.

Previously, the art has only provided for the analysis of a few known genes at a time by standard molecular biology techniques such as PCR, northern blot analysis, or other types of DNA probe analysis such as in situ hybridization. Each of these methods allows one to analyze the transcription of only known genes and/or small numbers of genes at a time. Nucl. Acids Res. 19, 7097-7104 (1991); Nucl. Acids Res. 18, 4833-42 (1990); Nucl. Acids Res. 18, 2789-92 (1989); European J. Neuroscience 2, 1063-1073 (1990); Analytical Biochem. 187, 364-73 (1990); Genet. Annals Techn. Appl. 7, 64-70 (1990); GATA 8(4), 129-33 (1991); Proc. Natl. Acad. Sci. USA 85, 1696-1700 (1988); Nucl. Acids Res. 19, 1954 (1991); Proc. Natl. Acad. Sci. USA 88, 1943-47 (1991); Nucl. Acids Res. 19, 6123-27 (1991); Proc. Natl. Acad. Sci. USA 85, 5738-42 (1988); Nucl. Acids Res. 16, 10937 (1988).

Studies of the number and types of genes whose transcription is induced or otherwise regulated during cell processes such as activation, differentiation, aging, viral transformation, morphogenesis, and mitosis have been pursued for many years, using a variety of methodologies. One of the earliest methods was to isolate and analyze levels of the proteins in a cell, tissue, organ system, or even organisms both before and after the process of interest. One method of analyzing multiple proteins in a sample is using 2-dimensional gel electrophoresis, wherein proteins can be, in principle, identified and quantified as individual bands, and ultimately reduced to a discrete signal. At present, 2-dimensional analysis only resolves approximately 15% of the proteins. In order to positively analyze those bands which are resolved, each band must be excised from the membrane and subjected to protein sequence analysis using Edman degradation. Unfortunately, most of the bands were present in quantities too small to obtain a reliable sequence, and many of those bands contained more than one discrete protein. An additional difficulty is that many of the proteins were blocked at the amino-terminus, further complicating the sequencing process.

Analyzing differentiation at the gene transcription level has overcome many of these disadvantages and drawbacks, since the power of recombinant DNA technology allows amplification of signals containing very small amounts of material. The most common method, called "hybridization subtraction," involves isolation of mRNA from the biological specimen before (B) and after (A) the developmental process of interest, transcribing one set of mRNA into cDNA, subtracting specimen B from specimen A (mRNA from cDNA) by hybridization, and constructing a cDNA library from the non-hybridizing mRNA fraction. Many different groups have used this strategy successfully, and a variety of procedures have been published and improved upon using this same basic scheme. Nucl. Acids Res. 19, 7097-7104 (1991); Nucl. Acids Res. 18, 4833-42 (1990); Nucl. Acids Res. 18, 2789-92 (1989); European J. Neuroscience 2, 1063-1073 (1990); Analytical Biochem. 187, 364-73 (1990); Genet. Annals Techn. Appl. 7, 64-70 (1990); GATA 8(4), 129-33 (1991); Proc. Natl. Acad. Sci. USA 85, 1696-1700 (1988); Nucl. Acids Res. 19, 1954 (1991); Proc. Natl. Acad. Sci. USA 88, 1943-47 (1991); Nucl. Acids Res. 19, 6123-27 (1991); Proc. Natl. Acad. Sci. USA 85, 5738-42 (1988); Nucl. Acids Res. 16, 10937 (1988).

Although each of these techniques have particular strengths and weaknesses, there are still some limitations and undesirable aspects of these methods: First, the time and effort required to construct such libraries is quite large. Typically, a trained molecular biologist might expect construction and characterization of such a library to require 3 to 6 months, depending on the level of skill, experience, and luck. Second, the resulting subtraction libraries are typically inferior to the libraries constructed by standard methodology. A typical conventional cDNA library should have a clone complexity of at least 10^6 clones, and an average insert size of 1-3 kB. In contrast, subtracted libraries can have complexities of 10^2 or 10^3 and average insert sizes of 0.2 kB. Therefore, there can be a significant loss of clone and sequence information associated with such libraries. Third, this

approach allows the researcher to capture only the genes induced in specimen A relative to specimen B, not vice-versa, nor does it easily allow comparison to a third specimen of interest (C). Fourth, this approach requires very large amounts (hundreds of micrograms) of "driver" mRNA (specimen B), which significantly limits the number and type of subtractions that are possible since many tissues and cells are very difficult to obtain in large quantities.

Fifth, the resolution of the subtraction is dependent upon the physical properties of DNA:DNA or RNA:DNA hybridization. The ability of a given sequence to find a hybridization match is dependent on its unique CoT value. The CoT value is a function of the number of copies (concentration) of the particular sequence, multiplied by the time of hybridization. It follows that for sequences which are abundant, hybridization events will occur very rapidly (low CoT value), while rare sequences will form duplexes at very high CoT values. CoT values which allow such rare sequences to form duplexes and therefore be effectively selected are difficult to achieve in a convenient time frame. Therefore, hybridization subtraction is simply not a useful technique with which to study relative levels of rare mRNA species. Sixth, this problem is further complicated by the fact that duplex formation is also dependent on the nucleotide base composition for a given sequence. Those sequences rich in G + C form stronger duplexes than those with high contents of A + T. Therefore, the former sequences will tend to be removed selectively by hybridization subtraction. Seventh, it is possible that hybridization between nonexact matches can occur. When this happens, the expression of a homologous gene may "mask" expression of a gene of interest, artificially skewing the results for that particular gene.

Matsubara and Okubo proposed using partial cDNA sequences to establish expression profiles of genes which could be used in functional analyses of the human genome. Matsubara and Okubo warned against using random priming, as

it creates multiple unique DNA fragments from individual mRNAs and may thus skew the analysis of the number of particular mRNAs per library. They sequenced randomly selected members from a 3'-directed cDNA library and
5 established the frequency of appearance of the various ESTs. They proposed comparing lists of ESTs from various cell types to classify genes. Genes expressed in many different cell types were labeled housekeepers and those selectively expressed in certain cells were labeled cell-
10 specific genes, even in the absence of the full sequence of the gene or the biological activity of the gene product.

The present invention avoids the drawbacks of the prior art by providing a method to quantify the relative abundance of multiple gene transcripts in a given
15 biological specimen by the use of high-throughput sequence-specific analysis of individual RNAs and/or their corresponding cDNAs.

The present invention offers several advantages over current protein discovery methods which attempt to isolate
20 individual proteins based upon biological effects. The method of the instant invention provides for detailed diagnostic comparisons of cell profiles revealing numerous changes in the expression of individual transcripts.

The instant invention provides several advantages over
25 current subtraction methods including a more complex library analysis (10^6 to 10^7 clones as compared to 10^3 clones) which allows identification of low abundance messages as well as enabling the identification of messages which either increase or decrease in abundance. These
30 large libraries are very routine to make in contrast to the libraries of previous methods. In addition, homologues can easily be distinguished with the method of the instant invention.

This method is very convenient because it organizes a
35 large quantity of data into a comprehensible, digestible format. The most significant differences are highlighted by electronic subtraction. In depth analyses are made more convenient.

The present invention provides several advantages over previous methods of electronic analysis of cDNA. The method is particularly powerful when more than 100 and preferably more than 1,000 gene transcripts are analyzed.

5 In such a case, new low-frequency transcripts are discovered and tissue typed.

High resolution analysis of gene expression can be used directly as a diagnostic profile or to identify disease-specific genes for the development of more classic
10 diagnostic approaches.

This process is defined as gene transcript frequency analysis. The resulting quantitative analysis of the gene transcripts is defined as comparative gene transcript analysis.

15 3. SUMMARY OF THE INVENTION

The invention is a method of analyzing a specimen containing gene transcripts comprising the steps of (a) producing a library of biological sequences; (b) generating a set of transcript sequences, where each of the transcript
20 sequences in said set is indicative of a different one of the biological sequences of the library; (c) processing the transcript sequences in a programmed computer (in which a database of reference transcript sequences indicative of reference sequences is stored), to generate an identified
25 sequence value for each of the transcript sequences, where each said identified sequence value is indicative of sequence annotation and a degree of match between one of the biological sequences of the library and at least one of the reference sequences; and (d) processing each said
30 identified sequence value to generate final data values indicative of the number of times each identified sequence value is present in the library.

The invention also includes a method of comparing two specimens containing gene transcripts. The first specimen
35 is processed as described above. The second specimen is used to produce a second library of biological sequences, which is used to generate a second set of transcript sequences, where each of the transcript sequences in the

In a further embodiment, the relative abundance of the gene transcripts in one cell type or tissue is compared with the relative abundance of gene transcript numbers in a second cell type or tissue in order to identify the differences and similarities.

In a further embodiment, the method includes a system for analyzing a library of biological sequences including a means for receiving a set of transcript sequences, where each of the transcript sequences is indicative of a different one of the biological sequences of the library; and a means for processing the transcript sequences in a computer system in which a database of reference transcript sequences indicative of reference sequences is stored, wherein the computer is programmed with software for generating an identified sequence value for each of the transcript sequences, where each said identified sequence value is indicative of a sequence annotation and the degree of match between a different one of the biological sequences of the library and at least one of the reference sequences, and for processing each said identified sequence value to generate final data values indicative of the number of times each identified sequence value is present in the library.

In essence, the invention is a method and system for quantifying the relative abundance of gene transcripts in a biological specimen. The invention provides a method for comparing the gene transcript image from two or more different biological specimens in order to distinguish between the two specimens and identify one or more genes which are differentially expressed between the two specimens. Thus, this gene transcript image and its comparison can be used as a diagnostic. One embodiment of the method generates high-throughput sequence-specific analysis of multiple RNAs or their corresponding cDNAs: a gene transcript image. Another embodiment of the method produces the gene transcript imaging analysis by the use of high-throughput cDNA sequence analysis. In addition, two or more gene transcript images can be compared and used to detect or diagnose a particular biological state, disease,

or condition which is correlated to the relative abundance of gene transcripts in a given cell or population of cells.

4. DESCRIPTION OF THE TABLES AND DRAWINGS

4.1. TABLES

5 Table 1 presents a detailed explanation of the letter codes utilized in Tables 2-5.

Table 2 lists the one hundred most common gene transcripts. It is a partial list of isolates from the HUVEC cDNA library prepared and sequenced as described
10 below. The left-hand column refers to the sequence's order of abundance in this table. The next column labeled "number" is the clone number of the first HUVEC sequence identification reference matching the sequence in the "entry" column number. Isolates that have not been
15 sequenced are not present in Table 2. The next column, labeled "N", indicates the total number of cDNAs which have the same degree of match with the sequence of the reference transcript in the "entry" column.

 The column labeled "entry" gives the NIH GENBANK locus
20 name, which corresponds to the library sequence numbers. The "s" column indicates in a few cases the species of the reference sequence. The code for column "s" is given in Table 1. The column labeled "descriptor" provides a plain English explanation of the identity of the sequence
25 corresponding to the NIH GENBANK locus name in the "entry" column.

Table 3 is a comparison of the top fifteen most abundant gene transcripts in normal monocytes and activated macrophage cells.

30 Table 4 is a detailed summary of library subtraction analysis summary comparing the THP-1 and human macrophage cDNA sequences. In Table 4, the same code as in Table 2 is used. Additional columns are for "bgfreq" (abundance number in the subtractant library), "rfend" (abundance
35 number in the target library) and "ratio" (the target abundance number divided by the subtractant abundance number). As is clear from perusal of the table, when the abundance number in the subtractant library is "0", the

target abundance number is divided by 0.05. This is a way of obtaining a result (not possible dividing by 0) and distinguishing the result from ratios of subtractant numbers of 1.

5 Table 5 is the computer program, written in source code, for generating gene transcript subtraction profiles.

Table 6 is a partial listing of database entries used in the electronic northern blot analysis as provided by the present invention.

10

4.2. BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a chart summarizing data collected and stored regarding the library construction portion of sequence preparation and analysis.

15 Figure 2 is a diagram representing the sequence of operations performed by "abundance sort" software in a class of preferred embodiments of the inventive method.

Figure 3 is a block diagram of a preferred embodiment of the system of the invention.

20 Figure 4 is a more detailed block diagram of the bioinformatics process from new sequence (that has already been sequenced but not identified) to printout of the transcript imaging analysis and the provision of database subscriptions.

25

5. DETAILED DESCRIPTION OF THE INVENTION

The present invention provides a method to compare the relative abundance of gene transcripts in different biological specimens by the use of high-throughput sequence-specific analysis of individual RNAs or their
30 corresponding cDNAs (or alternatively, of data representing other biological sequences). This process is denoted herein as gene transcript imaging. The quantitative analysis of the relative abundance for a set of gene transcripts is denoted herein as "gene transcript image
35 analysis" or "gene transcript frequency analysis". The present invention allows one to obtain a profile for gene transcription in any given population of cells or tissue from any type of organism. The invention can be applied to

obtain a profile of a specimen consisting of a single cell (or clones of a single cell), or of many cells, or of tissue more complex than a single cell and containing multiple cell types, such as liver.

- 5 The invention has significant advantages in the fields of diagnostics, toxicology and pharmacology, to name a few. A highly sophisticated diagnostic test can be performed on the ill patient in whom a diagnosis has not been made. A biological specimen consisting of the patient's fluids or
10 tissues is obtained, and the gene transcripts are isolated and expanded to the extent necessary to determine their identity. Optionally, the gene transcripts can be converted to cDNA. A sampling of the gene transcripts are subjected to sequence-specific analysis and quantified.
- 15 These gene transcript sequence abundances are compared against reference database sequence abundances including normal data sets for diseased and healthy patients. The patient has the disease(s) with which the patient's data set most closely correlates.

- 20 For example, gene transcript frequency analysis can be used to differentiate normal cells or tissues from diseased cells or tissues, just as it highlights differences between normal monocytes and activated macrophages in Table 3.

- In toxicology, a fundamental question is which tests
25 are most effective in predicting or detecting a toxic effect. Gene transcript imaging provides highly detailed information on the cell and tissue environment, some of which would not be obvious in conventional, less detailed screening methods. The gene transcript image is a more
30 powerful method to predict drug toxicity and efficacy. Similar benefits accrue in the use of this tool in pharmacology. The gene transcript image can be used selectively to look at protein categories which are expected to be affected, for example, enzymes which
35 detoxify toxins.

 In an alternative embodiment, comparative gene transcript frequency analysis is used to differentiate between cancer cells which respond to anti-cancer agents and those which do not respond. Examples of anti-cancer

agents are tamoxifen, vincristine, vinblastine, podophyllotoxins, etoposide, teniposide, cisplatin, biologic response modifiers such as interferon, Il-2, GM-CSF, enzymes, hormones and the like. This method also
5 provides a means for sorting the gene transcripts by functional category. In the case of cancer cells, transcription factors or other essential regulatory molecules are very important categories to analyze across different libraries.

10 In yet another embodiment, comparative gene transcript frequency analysis is used to differentiate between control liver cells and liver cells isolated from patients treated with experimental drugs like FIAU to distinguish between pathology caused by the underlying disease and that caused
15 by the drug.

In yet another embodiment, comparative gene transcript frequency analysis is used to differentiate between brain tissue from patients treated and untreated with lithium.

In a further embodiment, comparative gene transcript
20 frequency analysis is used to differentiate between cyclosporin and FK506-treated cells and normal cells.

In a further embodiment, comparative gene transcript frequency analysis is used to differentiate between virally infected (including HIV-infected) human cells and
25 uninfected human cells. Gene transcript frequency analysis is also used to rapidly survey gene transcripts in HIV-resistant, HIV-infected, and HIV-sensitive cells. Comparison of gene transcript abundance will indicate the success of treatment and/or new avenues to study.

30 In a further embodiment, comparative gene transcript frequency analysis is used to differentiate between bronchial lavage fluids from healthy and unhealthy patients with a variety of ailments.

In a further embodiment, comparative gene transcript
35 frequency analysis is used to differentiate between cell, plant, microbial and animal mutants and wild-type species. In addition, the transcript abundance program is adapted to permit the scientist to evaluate the transcription of one gene in many different tissues. Such comparisons could

identify deletion mutants which do not produce a gene product and point mutants which produce a less abundant or otherwise different message. Such mutations can affect basic biochemical and pharmacological processes, such as mineral nutrition and metabolism, and can be isolated by means known to those skilled in the art. Thus, crops with improved yields, pest resistance and other factors can be developed.

In a further embodiment, comparative gene transcript frequency analysis is used for an interspecies comparative analysis which would allow for the selection of better pharmacologic animal models. In this embodiment, humans and other animals (such as a mouse), or their cultured cells are treated with a specific test agent. The relative sequence abundance of each cDNA population is determined. If the animal test system is a good model, homologous genes in the animal cDNA population should change expression similarly to those in human cells. If side effects are detected with the drug, a detailed transcript abundance analysis will be performed to survey gene transcript changes. Models will then be evaluated by comparing basic physiological changes.

In a further embodiment, comparative gene transcript frequency analysis is used in a clinical setting to give a highly detailed gene transcript profile of a patient's cells or tissue (for example, a blood sample). In particular, gene transcript frequency analysis is used to give a high resolution gene expression profile of a diseased state or condition.

In the preferred embodiment, the method utilizes high-throughput cDNA sequencing to identify specific transcripts of interest. The generated cDNA and deduced amino acid sequences are then extensively compared with GENBANK and other sequence data banks as described below. The method offers several advantages over current protein discovery by two-dimensional gel methods which try to identify individual proteins involved in a particular biological effect. Here, detailed comparisons of profiles of activated and inactive cells reveal numerous changes in

the expression of individual transcripts. After it is determined if the sequence is an "exact" match, similar or a non-match, the sequence is entered into a database. Next, the numbers of copies of cDNA corresponding to each gene are tabulated. Although this can be done slowly and arduously, if at all, by human hand from a printout of all entries, a computer program is a useful and rapid way to tabulate this information. The numbers of cDNA copies (optionally divided by the total number of sequences in the data set) provides a picture of the relative abundance of transcripts for each corresponding gene. The list of represented genes can then be sorted by abundance in the cDNA population. A multitude of additional types of comparisons or dimensions are possible and are exemplified below.

An alternate method of producing a gene transcript image includes the steps of obtaining a mixture of test mRNA and providing a representative array of unique probes whose sequences are complementary to at least some of the test mRNAs. Next, a fixed amount of the test mRNA is added to the arrayed probes. The test mRNA is incubated with the probes for a sufficient time to allow hybrids of the test mRNA and probes to form. The mRNA-probe hybrids are detected and the quantity determined. The hybrids are identified by their location in the probe array. The quantity of each hybrid is summed to give a population number. Each hybrid quantity is divided by the population number to provide a set of relative abundance data termed a gene transcript image analysis.

30 6. EXAMPLES

The examples below are provided to illustrate the subject invention. These examples are provided by way of illustration and are not included for the purpose of limiting the invention.

35 6.1. TISSUE SOURCES AND CELL LINES

For analysis with the computer program claimed herein, biological sequences can be obtained from virtually any

source. Most popular are tissues obtained from the human body. Tissues can be obtained from any organ of the body, any age donor, any abnormality or any immortalized cell line. Immortal cell lines may be preferred in some instances because of their purity of cell type; other tissue samples invariably include mixed cell types. A special technique is available to take a single cell (for example, a brain cell) and harness the cellular machinery to grow up sufficient cDNA for sequencing by the techniques and analysis described herein (cf. U.S. Patent Nos. 5,021,335 and 5,168,038, which are incorporated by reference). The examples given herein utilized the following immortalized cell lines: monocyte-like U-937 cells, activated macrophage-like THP-1 cells, induced vascular endothelial cells (HUVEC cells) and mast cell-like HMC-1 cells.

The U-937 cell line is a human histiocytic lymphoma cell line with monocyte characteristics, established from malignant cells obtained from the pleural effusion of a patient with diffuse histiocytic lymphoma (Sundstrom, C. and Nilsson, K. (1976) Int. J. Cancer 17:565). U-937 is one of only a few human cell lines with the morphology, cytochemistry, surface receptors and monocyte-like characteristics of histiocytic cells. These cells can be induced to terminal monocytic differentiation and will express new cell surface molecules when activated with supernatants from human mixed lymphocyte cultures. Upon this type of in vitro activation, the cells undergo morphological and functional changes, including augmentation of antibody-dependent cellular cytotoxicity (ADCC) against erythroid and tumor target cells (one of the principal functions of macrophages). Activation of U-937 cells with phorbol 12-myristate 13-acetate (PMA) in vitro stimulates the production of several compounds, including prostaglandins, leukotrienes and platelet-activating factor (PAF), which are potent inflammatory mediators. Thus, U-937 is a cell line that is well suited for the identification and isolation of gene transcripts associated with normal monocytes.

The HUVEC cell line is a normal, homogeneous, well characterized, early passage endothelial cell culture from human umbilical vein (Cell Systems Corp., 12815 NE 124th Street, Kirkland, WA 98034). Only gene transcripts from induced, or treated, HUVEC cells were sequenced. One batch of 1×10^8 cells was treated for 5 hours with 1 U/ml rIL-1b and 100 ng/ml E.coli lipopolysaccharide (LPS) endotoxin prior to harvesting. A separate batch of 2×10^8 cells was treated at confluence with 4 U/ml TNF and 2 U/ml interferon-gamma (IFN-gamma) prior to harvesting.

THP-1 is a human leukemic cell line with distinct monocytic characteristics. This cell line was derived from the blood of a 1-year-old boy with acute monocytic leukemia (Tsuchiya, S. et al. (1980) Int. J. Cancer: 171-76). The following cytological and cytochemical criteria were used to determine the monocytic nature of the cell line: 1) the presence of alpha-naphthyl butyrate esterase activity which could be inhibited by sodium fluoride; 2) the production of lysozyme; 3) the phagocytosis of latex particles and sensitized SRBC (sheep red blood cells); and 4) the ability of mitomycin C-treated THP-1 cells to activate T-lymphocytes following ConA (concanavalin A) treatment. Morphologically, the cytoplasm contained small azurophilic granules and the nucleus was indented and irregularly shaped with deep folds. The cell line had Fc and C3b receptors, probably functioning in phagocytosis. THP-1 cells treated with the tumor promoter 12-o-tetradecanoyl-phorbol-13 acetate (TPA) stop proliferating and differentiate into macrophage-like cells which mimic native monocyte-derived macrophages in several respects. Morphologically, as the cells change shape, the nucleus becomes more irregular and additional phagocytic vacuoles appear in the cytoplasm. The differentiated THP-1 cells also exhibit an increased adherence to tissue culture plastic.

HMC-1 cells (a human mast cell line) were established from the peripheral blood of a Mayo Clinic patient with mast cell leukemia (Leukemia Res. (1988) 12:345-55). The cultured cells looked similar to immature cloned murine

mast cells, contained histamine, and stained positively for chloroacetate esterase, amino caproate esterase, eosinophil major basic protein (MBP) and tryptase. The HMC-1 cells have, however, lost the ability to synthesize normal IgE receptors. HMC-1 cells also possess a 10;16 translocation, present in cells initially collected by leukaphoresis from the patient and not an artifact of culturing. Thus, HMC-1 cells are a good model for mast cells.

6.2. CONSTRUCTION OF cDNA LIBRARIES

10 For inter-library comparisons, the libraries must be prepared in similar manners. Certain parameters appear to be particularly important to control. One such parameter is the method of isolating mRNA. It is important to use the same conditions to remove DNA and heterogeneous nuclear
15 RNA from comparison libraries. Size fractionation of cDNA must be carefully controlled. The same vector preferably should be used for preparing libraries to be compared. At the very least, the same type of vector (e.g., unidirectional vector) should be used to assure a valid
20 comparison. A unidirectional vector may be preferred in order to more easily analyze the output.

It is preferred to prime only with oligo dT unidirectional primer in order to obtain one only clone per mRNA transcript when obtaining cDNAs. However, it is
25 recognized that employing a mixture of oligo dT and random primers can also be advantageous because such a mixture results in more sequence diversity when gene discovery also is a goal. Similar effects can be obtained with DR2 (Clontech) and HXLOX (US Biochemical) and also vectors from
30 Invitrogen and Novagen. These vectors have two requirements. First, there must be primer sites for commercially available primers such as T3 or M13 reverse primers. Second, the vector must accept inserts up to 10 kB.

35 It also is important that the clones be randomly sampled, and that a significant population of clones is used. Data have been generated with 5,000 clones; however, if very rare genes are to be obtained and/or their relative

abundance determined, as many as 100,000 clones from a single library may need to be sampled. Size fractionation of cDNA also must be carefully controlled. Alternately, plaques can be selected, rather than clones.

5 Besides the Uni-ZAP™ vector system by Stratagene disclosed below, it is now believed that other similarly unidirectional vectors also can be used. For example, it is believed that such vectors include but are not limited to DR2 (Clontech), and HXLOX (U.S. Biochemical).

10 Preferably, the details of library construction (as shown in Figure 1) are collected and stored in a database for later retrieval relative to the sequences being compared. Fig. 1 shows important information regarding the library collaborator or cell or cDNA supplier,
15 pretreatment, biological source, culture, mRNA preparation and cDNA construction. Similarly detailed information about the other steps is beneficial in analyzing sequences and libraries in depth.

RNA must be harvested from cells and tissue samples
20 and cDNA libraries are subsequently constructed. cDNA libraries can be constructed according to techniques known in the art. (See, for example, Maniatis, T. et al. (1982) Molecular Cloning, Cold Spring Harbor Laboratory, New York). cDNA libraries may also be purchased. The U-937
25 cDNA library (catalog No. 937207) was obtained from Stratagene, Inc., 11099 M. Torrey Pines Rd., La Jolla, CA 92037.

The THP-1 cDNA library was custom constructed by Stratagene from THP-1 cells cultured 48 hours with 100 nm
30 TPA and 4 hours with 1 µg/ml LPS. The human mast cell HMC-1 cDNA library was also custom constructed by Stratagene from cultured HMC-1 cells. The HUVEC cDNA library was custom constructed by Stratagene from two batches of induced HUVEC cells which were separately processed.

35 Essentially, all the libraries were prepared in the same manner. First, poly(A+)RNA (mRNA) was purified. For the U-937 and HMC-1 RNA, cDNA synthesis was only primed with oligo dT. For the THP-1 and HUVEC RNA, cDNA synthesis was primed separately with both oligo dT and random

hexamers, and the two cDNA libraries were treated separately. Synthetic adaptor oligonucleotides were ligated onto cDNA ends enabling its insertion into the Uni-Zap™ vector system (Stratagene), allowing high efficiency
5 unidirectional (sense orientation) lambda library construction and the convenience of a plasmid system with blue-white color selection to detect clones with cDNA insertions. Finally, the two libraries were combined into a single library by mixing equal numbers of bacteriophage.
10 The libraries can be screened with either DNA probes or antibody probes and the pBluescript® phagemid (Stratagene) can be rapidly excised in vivo. The phagemid allows the use of a plasmid system for easy insert characterization, sequencing, site-directed mutagenesis,
15 the creation of unidirectional deletions and expression of fusion proteins. The custom-constructed library phage particles were infected into E. coli host strain XL1-Blue® (Stratagene), which has a high transformation efficiency, increasing the probability of obtaining rare, under-
20 represented clones in the cDNA library.

6.3. ISOLATION OF cDNA CLONES

The phagemid forms of individual cDNA clones were obtained by the in vivo excision process, in which the host bacterial strain was coinfecting with both the lambda
25 library phage and an f1 helper phage. Proteins derived from both the library-containing phage and the helper phage nicked the lambda DNA, initiated new DNA synthesis from defined sequences on the lambda target DNA and created a smaller, single stranded circular phagemid DNA molecule
30 that included all DNA sequences of the pBluescript® plasmid and the cDNA insert. The phagemid DNA was secreted from the cells and purified, then used to re-infect fresh host cells, where the double stranded phagemid DNA was produced. Because the phagemid carries the gene for beta-lactamase,
35 the newly-transformed bacteria are selected on medium containing ampicillin.

Phagemid DNA was purified using the Magic Minipreps™ DNA Purification System (Promega catalogue #A7100. Promega

Corp., 2800 Woods Hollow Rd., Madison, WI 53711). This small-scale process provides a simple and reliable method for lysing the bacterial cells and rapidly isolating purified phagemid DNA using a proprietary DNA-binding resin. The DNA was eluted from the purification resin already prepared for DNA sequencing and other analytical manipulations.

Phagemid DNA was also purified using the QIAwell-8 Plasmid Purification System from QIAGEN® DNA Purification System (QIAGEN Inc., 9259 Eton Ave., Chatsworth, CA 91311). This product line provides a convenient, rapid and reliable high-throughput method for lysing the bacterial cells and isolating highly purified phagemid DNA using QIAGEN anion-exchange resin particles with EMPORE™ membrane technology from 3M in a multiwell format. The DNA was eluted from the purification resin already prepared for DNA sequencing and other analytical manipulations.

An alternate method of purifying phagemid has recently become available. It utilizes the Miniprep Kit (Catalog No. 77468, available from Advanced Genetic Technologies Corp., 19212 Orbit Drive, Gaithersburg, Maryland). This kit is in the 96-well format and provides enough reagents for 960 purifications. Each kit is provided with a recommended protocol, which has been employed except for the following changes. First, the 96 wells are each filled with only 1 ml of sterile terrific broth with carbenicillin at 25 mg/L and glycerol at 0.4%. After the wells are inoculated, the bacteria are cultured for 24 hours and lysed with 60 µl of lysis buffer. A centrifugation step (2900 rpm for 5 minutes) is performed before the contents of the block are added to the primary filter plate. The optional step of adding isopropanol to TRIS buffer is not routinely performed. After the last step in the protocol, samples are transferred to a Beckman 96-well block for storage.

Another new DNA purification system is the WIZARD™ product line which is available from Promega (catalog No. A7071) and may be adaptable to the 96-well format.

6.4. SEQUENCING OF cDNA CLONES

The cDNA inserts from random isolates of the U-937 and THP-1 libraries were sequenced in part. Methods for DNA sequencing are well known in the art. Conventional enzymatic methods employ DNA polymerase Klenow fragment, Sequenase™ or Tag polymerase to extend DNA chains from an oligonucleotide primer annealed to the DNA template of interest. Methods have been developed for the use of both single- and double-stranded templates. The chain termination reaction products are usually electrophoresed on urea-acrylamide gels and are detected either by autoradiography (for radionuclide-labeled precursors) or by fluorescence (for fluorescent-labeled precursors). Recent improvements in mechanized reaction preparation, sequencing and analysis using the fluorescent detection method have permitted expansion in the number of sequences that can be determined per day (such as the Applied Biosystems 373 and 377 DNA sequencer, Catalyst 800). Currently with the system as described, read lengths range from 250 to 400 bases and are clone dependent. Read length also varies with the length of time the gel is run. In general, the shorter runs tend to truncate the sequence. A minimum of only about 25 to 50 bases is necessary to establish the identification and degree of homology of the sequence. Gene transcript imaging can be used with any sequence-specific method, including, but not limited to hybridization, mass spectroscopy, capillary electrophoresis and 505 gel electrophoresis.

30 6.5. HOMOLOGY SEARCHING OF cDNA CLONE AND DEDUCED PROTEIN (and Subsequent Steps)

Using the nucleotide sequences derived from the cDNA clones as query sequences (sequences of a Sequence Listing), databases containing previously identified sequences are searched for areas of homology (similarity). Examples of such databases include Genbank and EMBL. We next describe examples of two homology search algorithms that can be used, and then describe the subsequent computer-implemented steps to be performed in accordance with preferred embodiments of the invention.

In the following description of the computer-implemented steps of the invention, the word "library" denotes a set (or population) of biological specimen nucleic acid sequences. A "library" can consist of cDNA sequences, RNA sequences, or the like, which characterize a biological specimen. The biological specimen can consist of cells of a single human cell type (or can be any of the other above-mentioned types of specimens). We contemplate that the sequences in a library have been determined so as to accurately represent or characterize a biological specimen (for example, they can consist of representative cDNA sequences from clones of RNA taken from a single human cell).

In the following description of the computer-implemented steps of the invention, the expression "database" denotes a set of stored data which represent a collection of sequences, which in turn represent a collection of biological reference materials. For example, a database can consist of data representing many stored cDNA sequences which are in turn representative of human cells infected with various viruses, cells of humans of various ages, cells from different mammalian species, and so on.

In preferred embodiments, the invention employs a computer programmed with software (to be described) for performing the following steps:

(a) processing data indicative of a library of cDNA sequences (generated as a result of high-throughput cDNA sequencing or other method) to determine whether each sequence in the library matches a DNA sequence of a reference database of DNA sequences (and if so, identifying the reference database entry which matches the sequence and indicating the degree of match between the reference sequence and the library sequence) and assigning an identified sequence value based on the sequence annotation and degree of match to each of the sequences in the library;

(b) for some or all entries of the database, tabulating the number of matching identified sequence

values in the library (Although this can be done by human hand from a printout of all entries, we prefer to perform this step using computer software to be described below.), thereby generating a set of final data values or "abundance numbers"; and

(c) if the libraries are different sizes, dividing each abundance number by the total number of sequences in the library, to obtain a relative abundance number for each identified sequence value (i.e., a relative abundance of each gene transcript).

The list of identified sequence values (or genes corresponding thereto) can then be sorted by abundance in the cDNA population. A multitude of additional types of comparisons or dimensions are possible.

For example (to be described below in greater detail), steps (a) and (b) can be repeated for two different libraries (sometimes referred to as a "target" library and a "subtractant" library). Then, for each identified sequence value (or gene transcript), a "ratio" value is obtained by dividing the abundance number (for that identified sequence value) for the target library, by the abundance number (for that identified sequence value) for the subtractant library.

In fact, subtraction may be carried out on multiple libraries. It is possible to add the transcripts from several libraries (for example, three) and then to divide them by another set of transcripts from multiple libraries (again, for example, three). Notation for this operation may be abbreviated as $(A+B+C) / (D+E+F)$, where the capital letters each indicate an entire library. Optionally the abundance numbers of transcripts in the summed libraries may be divided by the total sample size before subtraction.

Unlike standard hybridization technology which permits a single subtraction of two libraries, once one has processed a set or library transcript sequences and stored them in the computer, any number of subtractions can be performed on the library. For example, by this method, ratio values can be obtained by dividing relative abundance

values in a first library by corresponding values in a second library and vice versa.

In variations on step (a), the library consists of nucleotide sequences derived from cDNA clones. Examples of
5 databases which can be searched for areas of homology (similarity) in step (a) include the commercially available databases known as Genbank (NIH) EMBL (European Molecular Biology Labs, Germany), and GENESEQ (Intelligenetics, Mountain View, California).

10 One homology search algorithm which can be used to implement step (a) is the algorithm described in the paper by D.J. Lipman and W.R. Pearson, entitled "Rapid and Sensitive Protein Similarity Searches," Science, 227:1435 (1985). In this algorithm, the homologous regions are
15 searched in a two-step manner. In the first step, the highest homologous regions are determined by calculating a matching score using a homology score table. The parameter "Ktup" is used in this step to establish the minimum window size to be shifted for comparing two sequences. Ktup also
20 sets the number of bases that must match to extract the highest homologous region among the sequences. In this step, no insertions or deletions are applied and the homology is displayed as an initial (INIT) value.

In the second step, the homologous regions are aligned
25 to obtain the highest matching score by inserting a gap in order to add a probable deleted portion. The matching score obtained in the first step is recalculated using the homology score Table and the insertion score Table to an optimized (OPT) value in the final output.

30 DNA homologies between two sequences can be examined graphically using the Harr method of constructing dot matrix homology plots (Needleman, S.B. and Wunsch, C.O., J. Mom. Biol 48:443 (1970)). This method produces a two-dimensional plot which can be useful in determining
35 regions of homology versus regions of repetition.

However, in a class of preferred embodiments, step (a) is implemented by processing the library data in the commercially available computer program known as the INHERIT 670 Sequence Analysis System, available from

Applied Biosystems Inc. (Foster City, California), including the software known as the Factura software (also available from Applied Biosystems Inc.). The Factura program preprocesses each library sequence to "edit out" portions thereof which are not likely to be of interest, such as the vector used to prepare the library. Additional sequences which can be edited out or masked (ignored by the search tools) include but are not limited to the polyA tail and repetitive GAG and CCC sequences. A low-end search program can be written to mask out such "low-information" sequences, or programs such as BLAST can ignore the low-information sequences.

In the algorithm implemented by the INHERIT 670 Sequence Analysis System, the Pattern Specification Language (developed by TRW Inc.) is used to determine regions of homology. "There are three parameters that determine how INHERIT analysis runs sequence comparisons: window size, window offset and error tolerance. Window size specifies the length of the segments into which the query sequence is subdivided. Window offset specifies where to start the next segment [to be compared], counting from the beginning of the previous segment. Error tolerance specifies the total number of insertions, deletions and/or substitutions that are tolerated over the specified word length. Error tolerance may be set to any integer between 0 and 6. The default settings are window tolerance=20, window offset=10 and error tolerance=3." INHERIT Analysis Users Manual, pp.2-15. Version 1.0, Applied Biosystems, Inc., October 1991.

Using a combination of these three parameters, a database (such as a DNA database) can be searched for sequences containing regions of homology and the appropriate sequences are scored with an initial value. Subsequently, these homologous regions are examined using dot matrix homology plots to determine regions of homology versus regions of repetition. Smith-Waterman alignments can be used to display the results of the homology search. The INHERIT software can be executed by a Sun computer system programmed with the UNIX operating system.

Search alternatives to INHERIT include the BLAST program, GCG (available from the Genetics Computer Group, WI) and the Dasher program (Temple Smith, Boston University, Boston, MA). Nucleotide sequences can be
5 searched against Genbank, EMBL or custom databases such as GENESEQ (available from Intelligenetics, Mountain View, CA) or other databases for genes. In addition, we have searched some sequences against our own in-house database.

In preferred embodiments, the transcript sequences are
10 analyzed by the INHERIT software for best conformance with a reference gene transcript to assign a sequence identifier and assigned the degree of homology, which together are the identified sequence value and are input into, and further processed by, a Macintosh personal computer (available from
15 Apple) programmed with an "abundance sort and subtraction analysis" computer program (to be described below).

Prior to the abundance sort and subtraction analysis program (also denoted as the "abundance sort" program), identified sequences from the cDNA clones are assigned
20 value (according to the parameters given above) by degree of match according to the following categories: "exact" matches (regions with a high degree of identity), homologous human matches (regions of high similarity, but not "exact" matches), homologous non-human matches (regions
25 of high similarity present in species other than human), or non matches (no significant regions of homology to previously identified nucleotide sequences stored in the form of the database). Alternately, the degree of match can be a numeric value as described below.

30 With reference again to the step of identifying matches between reference sequences and database entries, protein and peptide sequences can be deduced from the nucleic acid sequences. Using the deduced polypeptide sequence, the match identification can be performed in a
35 manner analogous to that done with cDNA sequences. A protein sequence is used as a query sequence and compared to the previously identified sequences contained in a database such as the Swiss/Prot, PIR and the NBRF Protein database to find homologous proteins. These proteins are

initially scored for homology using a homology score Table (Orcutt, B.C. and Dayoff, M.O. Scoring Matrices, PIR Report MAT - 0285 (February 1985)) resulting in an INIT score. The homologous regions are aligned to obtain the
5 highest matching scores by inserting a gap which adds a probable deleted portion. The matching score is recalculated using the homology score Table and the insertion score Table resulting in an optimized (OPT) score. Even in the absence of knowledge of the proper
10 reading frame of an isolated sequence, the above-described protein homology search may be performed by searching all 3 reading frames.

Peptide and protein sequence homologies can also be ascertained using the INHERIT 670 Sequence Analysis System
15 in an analogous way to that used in DNA sequence homologies. Pattern Specification Language and parameter windows are used to search protein databases for sequences containing regions of homology which are scored with an initial value. Subsequent display in a dot-matrix homology
20 plot shows regions of homology versus regions of repetition. Additional search tools that are available to use on pattern search databases include PLsearch Blocks (available from Henikoff & Henikoff, University of Washington, Seattle), Dasher and GCG. Pattern search
25 databases include, but are not limited to, Protein Blocks (available from Henikoff & Henikoff, University of Washington, Seattle), Brookhaven Protein (available from the Brookhaven National Laboratory, Brookhaven, MA), PROSITE (available from Amos Bairoch, University of Geneva,
30 Switzerland), ProDom (available from Temple Smith, Boston University), and PROTEIN MOTIF FINGERPRINT (available from University of Leeds, United Kingdom).

The ABI Assembler application software, part of the INHERIT DNA analysis system (available from Applied
35 Biosystems, Inc., Foster City, CA), can be employed to create and manage sequence assembly projects by assembling data from selected sequence fragments into a larger sequence. The Assembler software combines two advanced computer technologies which maximize the ability to

assemble sequenced DNA fragments into Assemblages, a special grouping of data where the relationships between sequences are shown by graphic overlap, alignment and statistical views. The process is based on the

5 Meyers-Kececiloglu model of fragment assembly (INHERIT™ Assembler User's Manual, Applied Biosystems, Inc., Foster City, CA), and uses graph theory as the foundation of a very rigorous multiple sequence alignment engine for assembling DNA sequence fragments. Other assembly programs

10 that can be used include MEGALIGN (available from DNASTAR Inc., Madison, WI), Dasher and STADEN (available from Roger Staden, Cambridge, England).

Next, with reference to Fig. 2, we describe in more detail the "abundance sort" program which implements above-

15 mentioned "step (b)" to tabulate the number of sequences of the library which match each database entry (the "abundance number" for each database entry).

Fig. 2 is a flow chart of a preferred embodiment of the abundance sort program. A source code listing of this

20 embodiment of the abundance sort program is set forth in Table 5. In the Table 5 implementation, the abundance sort program is written using the FoxBASE programming language commercially available from Microsoft Corporation. Although FoxBASE was the program chosen for the first

25 iteration of this technology, it should not be considered limiting. Many other programming languages, Sybase being a particularly desirable alternative, can also be used, as will be obvious to one with ordinary skill in the art. The subroutine names specified in Fig. 2 correspond to

30 subroutines listed in Table 5.

With reference again to Fig. 2, the "Identified Sequences" are transcript sequences representing each sequence of the library and a corresponding identification of the database entry (if any) which it matches. In other

35 words, the "Identified Sequences" are transcript sequences representing the output of above-discussed "step (a)."

Fig. 3 is a block diagram of a system for implementing the invention. The Fig. 3 system includes library generation unit 2 which generates a library and asserts an

output stream of transcript sequences indicative of the biological sequences comprising the library. Programmed processor 4 receives the data stream output from unit 2 and processes this data in accordance with above-discussed

5 "step (a)" to generate the Identified Sequences. Processor 4 can be a processor programmed with the commercially available computer program known as the INHERIT 670 Sequence Analysis System and the commercially available computer program known as the Factura program (both

10 available from Applied Biosystems Inc.) and with the UNIX operating system.

Still with reference to Fig. 3, the Identified Sequences are loaded into processor 6 which is programmed with the abundance sort program. Processor 6 generates the

15 Final Transcript sequences indicated in both Figs. 2 and 3. Fig. 4 shows a more detailed block diagram of a planned relational computer system, including various searching techniques which can be implemented, along with an assortment of databases to query against.

20 With reference to Fig. 2, the abundance sort program first performs an operation known as "Tempnum" on the Identified Sequences, to discard all of the Identified Sequences except those which match database entries of selected types. For example, the Tempnum process can

25 select Identified Sequences which represent matches of the following types with database entries (see above for definition): "exact" matches, human "homologous" matches, "other species" matches representing genes present in species other than human), "no" matches (no significant

30 regions of homology with database entries representing previously identified nucleotide sequences), "I" matches (Incyte for not previously known DNA sequences), or "X" matches (matches ESTs in reference database). This eliminates the U, S, M, V, A, R and D sequence (see Table 1

35 for definitions).

The identified sequence values selected during the "Tempnum" process then undergo a further selection (weeding out) operation known as "Tempred." This operation can, for

example, discard all identified sequence values representing matches with selected database entries.

The identified sequence values selected during the "Tempred" process are then classified according to library, during the "Tempdesig" operation. It is contemplated that the "Identified Sequences" can represent sequences from a single library, or from two or more libraries.

Consider first the case that the identified sequence values represent sequences from a single library. In this case, all the identified sequence values determined during "Tempred" undergo sorting in the "Templib" operation, further sorting in the "Libsort" operation, and finally additional sorting in the "Temptarsort" operation. For example, these three sorting operations can sort the identified sequences in order of decreasing "abundance number" (to generate a list of decreasing abundance numbers, each abundance number corresponding to a unique identified sequence entry, or several lists of decreasing abundance numbers, with the abundance numbers in each list corresponding to database entries of a selected type) with redundancies eliminated from each sorted list. In this case, the operation identified as "Cruncher" can be bypassed, so that the "Final Data" values are the organized transcript sequences produced during the "Temptarsort" operation.

We next consider the case that the transcript sequences produced during the "Tempred" operation represent sequences from two libraries (which we will denote the "target" library and the "subtractant" library). For example, the target library may consist of cDNA sequences from clones of a diseased cell, while the subtractant library may consist of cDNA sequences from clones of the diseased cell after treatment by exposure to a drug. For another example, the target library may consist of cDNA sequences from clones of a cell type from a young human, while the subtractant library may consist of cDNA sequences from clones of the same cell type from the same human at different ages.

In this case, the "Tempdesig" operation routes all transcript sequences representing the target library for processing in accordance with "Templib" (and then "Libsort" and "Temptarsort"), and routes all transcript sequences
5 representing the subtractant library for processing in accordance with "Tempsub" (and then "Subsort" and "Tempsubsort"). For example, the consecutive "Templib," "Libsort," and "Temptarsort" sorting operations sort identified sequences from the target library in order of
10 decreasing abundance number (to generate a list of decreasing abundance numbers, each abundance number corresponding to a database entry, or several lists of decreasing abundance numbers, with the abundance numbers in each list corresponding to database entries of a selected
15 type) with redundancies eliminated from each sorted list. The consecutive "Tempsub," "Subsort," and "Tempsubsort" sorting operations sort identified sequences from the subtractant library in order of decreasing abundance number (to generate a list of decreasing abundance numbers, each
20 abundance number corresponding to a database entry, or several lists of decreasing abundance numbers, with the abundance numbers in each list corresponding to database entries of a selected type) with redundancies eliminated from each sorted list.

25 The transcript sequences output from the "Temptarsort" operation typically represent sorted lists from which a histogram could be generated in which position along one (e.g., horizontal) axis indicates abundance number (of target library sequences), and position along another
30 (e.g., vertical) axis indicates identified sequence value (e.g., human or non-human gene type). Similarly, the transcript sequences output from the "Tempsubsort" operation typically represent sorted lists from which a histogram could be generated in which position along one
35 (e.g., horizontal) axis indicates abundance number (of subtractant library sequences), and position along another (e.g., vertical) axis indicates identified sequence value (e.g., human or non-human gene type).

The transcript sequences (sorted lists) output from the Tempsubsort and Temparsort sorting operations are combined during the operation identified as "Cruncher." The "Cruncher" process identifies pairs of corresponding target and subtractant abundance numbers (both representing the same identified sequence value), and divides one by the other to generate a "ratio" value for each pair of corresponding abundance numbers, and then sorts the ratio values in order of decreasing ratio value. The data output from the "Cruncher" operation (the Final Transcript sequence in Fig. 2) is typically a sorted list from which a histogram could be generated in which position along one axis indicates the size of a ratio of abundance numbers (for corresponding identified sequence values from target and subtractant libraries) and position along another axis indicates identified sequence value (e.g., gene type).

Preferably, prior to obtaining a ratio between the two library abundance values, the Cruncher operation also divides each ratio value by the total number of sequences in one or both of the target and subtractant libraries. The resulting lists of "relative" ratio values generated by the Cruncher operation are useful for many medical, scientific, and industrial applications. Also preferably, the output of the Cruncher operation is a set of lists, each list representing a sequence of decreasing ratio values for a different selected subset (e.g. protein family) of database entries.

In one example, the abundance sort program of the invention tabulates for a library the numbers of mRNA transcripts corresponding to each gene identified in a database. These numbers are divided by the total number of clones sampled. The results of the division reflect the relative abundance of the mRNA transcripts in the cell type or tissue from which they were obtained. Obtaining this final data set is referred to herein as "gene transcript image analysis." The resulting subtracted data show exactly what proteins and genes are upregulated and downregulated in highly detailed complexity.

6.6. HUVEC cDNA LIBRARY

Table 2 is an abundance table listing the various gene transcripts in an induced HUVEC library. The transcripts are listed in order of decreasing abundance. This computerized sorting simplifies analysis of the tissue and speeds identification of significant new proteins which are specific to this cell type. This type of endothelial cell lines tissues of the cardiovascular system, and the more that is known about its composition, particularly in response to activation, the more choices of protein targets become available to affect in treating disorders of this tissue, such as the highly prevalent atherosclerosis.

6.7. MONOCYTE-CELL AND MAST-CELL cDNA LIBRARIES

Tables 3 and 4 show truncated comparisons of two libraries. In Tables 3 and 4 the "normal monocytes" are the HMC-1 cells, and the "activated macrophages" are the THP-1 cells pretreated with PMA and activated with LPS. Table 3 lists in descending order of abundance the most abundant gene transcripts for both cell types. With only 15 gene transcripts from each cell type, this table permits quick, qualitative comparison of the most common transcripts. This abundance sort, with its convenient side-by-side display, provides an immediately useful research tool. In this example, this research tool discloses that 1) only one of the top 15 activated macrophage transcripts is found in the top 15 normal monocyte gene transcripts (poly A binding protein); and 2) a new gene transcript (previously unreported in other databases) is relatively highly represented in activated macrophages but is not similarly prominent in normal macrophages. Such a research tool provides researchers with a short-cut to new proteins, such as receptors, cell-surface and intracellular signalling molecules, which can serve as drug targets in commercial drug screening programs. Such a tool could save considerable time over that consumed by a hit and miss discovery program aimed at identifying important proteins in and around cells, because those proteins carrying out everyday cellular functions and

represented as steady state mRNA are quickly eliminated from further characterization.

This illustrates how the gene transcript profiles change with altered cellular function. Those skilled in the art know that the biochemical composition of cells also changes with other functional changes such as cancer, including cancer's various stages, and exposure to toxicity. A gene transcript subtraction profile such as in Table 3 is useful as a first screening tool for such gene expression and protein studies.

6.8. SUBTRACTION ANALYSIS OF NORMAL MONOCYTE-CELL AND ACTIVATED MONOCYTE CELL cDNA LIBRARIES

Once the cDNA data are in the computer, the computer program as disclosed in Table 5 was used to obtain ratios of all the gene transcripts in the two libraries discussed in Example 6.7, and the gene transcripts were sorted by the descending values of their ratios. If a gene transcript is not represented in one library, that gene transcript's abundance is unknown but appears to be less than 1. As an approximation -- and to obtain a ratio, which would not be possible if the unrepresented gene were given an abundance of zero -- genes which are represented in only one of the two libraries are assigned an abundance of 1/2. Using 1/2 for unrepresented clones increases the relative importance of "turned-on" and "turned-off" genes, whose products would be drug candidates. The resulting print-out is called a subtraction table and is an extremely valuable screening method, as is shown by the following data.

Table 4 is a subtraction table, in which the normal monocyte library was electronically "subtracted" from the activated macrophage library. This table highlights most effectively the changes in abundance of the gene transcripts by activation of macrophages. Even among the first 20 gene transcripts listed, there are several unknown gene transcripts. Thus, electronic subtraction is a useful tool with which to assist researchers in identifying much more quickly the basic biochemical changes between two cell types. Such a tool can save universities and pharmaceutical companies which spend billions of dollars on

research valuable time and laboratory resources at the early discovery stage and can speed up the drug development cycle, which in turn permits researchers to set up drug screening programs much earlier. Thus, this research tool
5 provides a way to get new drugs to the public faster and more economically.

Also, such a subtraction table can be obtained for patient diagnosis. An individual patient sample (such as monocytes obtained from a biopsy or blood sample) can be
10 compared with data provided herein to diagnose conditions associated with macrophage activation.

Table 4 uncovered many new gene transcripts (labeled Incyte clones). Note that many genes are turned on in the activated macrophage (i.e., the monocyte had a 0 in the
15 bgfreq column). This screening method is superior to other screening techniques, such as the western blot, which are incapable of uncovering such a multitude of discrete new gene transcripts.

The subtraction-screening technique has also uncovered
20 a high number of cancer gene transcripts (oncogenes rho, ETS2, rab-2 ras, YPT1-related, and acute myeloid leukemia mRNA) in the activated macrophage. These transcripts may be attributed to the use of immortalized cell lines and are inherently interesting for that reason. This screening
25 technique offers a detailed picture of upregulated transcripts including oncogenes, which helps explain why anti-cancer drugs interfere with the patient's immunity mediated by activated macrophages. Armed with knowledge gained from this screening method, those skilled in the art
30 can set up more targeted, more effective drug screening programs to identify drugs which are differentially effective against 1) both relevant cancers and activated macrophage conditions with the same gene transcript profile; 2) cancer alone; and 3) activated macrophage
35 conditions.

Smooth muscle senescent protein (22 kd) was upregulated in the activated macrophage, which indicates that it is a candidate to block in controlling inflammation.

6.9. SUBTRACTION ANALYSIS OF NORMAL LIVER CELLS AND HEPATITIS INFECTED LIVER CELL cDNA LIBRARIES

In this example, rats are exposed to hepatitis virus and maintained in the colony until they show definite signs of hepatitis. Of the rats diagnosed with hepatitis, one half of the rats are treated with a new anti-hepatitis agent (AHA). Liver samples are obtained from all rats before exposure to the hepatitis virus and at the end of AHA treatment or no treatment. In addition, liver samples can be obtained from rats with hepatitis just prior to AHA treatment.

The liver tissue is treated as described in Examples 6.2 and 6.3 to obtain mRNA and subsequently to sequence cDNA. The cDNA from each sample are processed and analyzed for abundance according to the computer program in Table 5. The resulting gene transcript images of the cDNA provide detailed pictures of the baseline (control) for each animal and of the infected and/or treated state of the animals. cDNA data for a group of samples can be combined into a group summary gene transcript profile for all control samples, all samples from infected rats and all samples from AHA-treated rats.

Subtractions are performed between appropriate individual libraries and the grouped libraries. For individual animals, control and post-study samples can be subtracted. Also, if samples are obtained before and after AHA treatment, that data from individual animals and treatment groups can be subtracted. In addition, the data for all control samples can be pooled and averaged. The control average can be subtracted from averages of both post-study AHA and post-study non-AHA cDNA samples. If pre- and post-treatment samples are available, pre- and post-treatment samples can be compared individually (or electronically averaged) and subtracted.

These subtraction tables are used in two general ways. First, the differences are analyzed for gene transcripts which are associated with continuing hepatic deterioration or healing. The subtraction tables are tools to isolate the effects of the drug treatment from the underlying basic pathology of hepatitis. Because hepatitis affects many

parameters, additional liver toxicity has been difficult to detect with only blood tests for the usual enzymes. The gene transcript profile and subtraction provides a much more complex biochemical picture which researchers have
5 needed to analyze such difficult problems.

Second, the subtraction tables provide a tool for identifying clinical markers, individual proteins or other biochemical determinants which are used to predict and/or evaluate a clinical endpoint, such as disease, improvement
10 due to the drug, and even additional pathology due to the drug. The subtraction tables specifically highlight genes which are turned on or off. Thus, the subtraction tables provide a first screen for a set of gene transcript candidates for use as clinical markers. Subsequently,
15 electronic subtractions of additional cell and tissue libraries reveal which of the potential markers are in fact found in different cell and tissue libraries. Candidate gene transcripts found in additional libraries are removed from the set of potential clinical markers. Then, tests of
20 blood or other relevant samples which are known to lack and have the relevant condition are compared to validate the selection of the clinical marker. In this method, the particular physiologic function of the protein transcript need not be determined to qualify the gene transcript as a
25 clinical marker.

6.10. ELECTRONIC NORTHERN BLOT

One limitation of electronic subtraction is that it is difficult to compare more than a pair of images at once. Once particular individual gene products are identified as
30 relevant to further study (via electronic subtraction or other methods), it is useful to study the expression of single genes in a multitude of different tissues. In the lab, the technique of "Northern" blot hybridization is used for this purpose. In this technique, a single cDNA, or a
35 probe corresponding thereto, is labeled and then hybridized against a blot containing RNA samples prepared from a multitude of tissues or cell types. Upon autoradiography,

second set is indicative of one of the biological sequences of the second library. Then the second set of transcript sequences is processed in a programmed computer to generate a second set of identified sequence values, namely the

5 further identified sequence values, each of which is indicative of a sequence annotation and includes a degree of match between one of the biological sequences of the second library and at least one of the reference sequences. The further identified sequence values are processed to

10 generate further final data values indicative of the number of times each further identified sequence value is present in the second library. The final data values from the first specimen and the further identified sequence values from the second specimen are processed to generate ratios

15 of transcript sequences, which indicate the differences in the number of gene transcripts between the two specimens.

In a further embodiment, the method includes quantifying the relative abundance of mRNA in a biological specimen by (a) isolating a population of mRNA transcripts

20 from a biological specimen; (b) identifying genes from which the mRNA was transcribed by a sequence-specific method; (c) determining the numbers of mRNA transcripts corresponding to each of the genes; and (d) using the mRNA transcript numbers to determine the relative abundance of

25 mRNA transcripts within the population of mRNA transcripts.

Also disclosed is a method of producing a gene transcript image analysis by first obtaining a mixture of mRNA, from which cDNA copies are made. The cDNA is inserted into a suitable vector which is used to transfect

30 suitable host strain cells which are plated out and permitted to grow into clones, each clone representing a unique mRNA. A representative population of clones transfected with cDNA is isolated. Each clone in the population is identified by a sequence-specific method

35 which identifies the gene from which the unique mRNA was transcribed. The number of times each gene is identified to a clone is determined to evaluate gene transcript abundance. The genes and their abundances are listed in order of abundance to produce a gene transcript image.

the pattern of expression of that particular gene, one at a time, can be quantitated in all the included samples.

In contrast, a further embodiment of this invention is the computerized form of this process, termed here
5 "electronic northern blot." In this variation, a single gene is queried for expression against a multitude of prepared and sequenced libraries present within the database. In this way, the pattern of expression of any single candidate gene can be examined instantaneously and
10 effortlessly. More candidate genes can thus be scanned, leading to more frequent and fruitfully relevant discoveries. The computer program included as Table 5 includes a program for performing this function, and Table 6 is a partial listing of entries of the database used in
15 the electronic northern blot analysis.

6.11. PHASE I CLINICAL TRIALS

Based on the establishment of safety and effectiveness in the above animal tests, Phase I clinical tests are undertaken. Normal patients are subjected to the usual
20 preliminary clinical laboratory tests. In addition, appropriate specimens are taken and subjected to gene transcript analysis. Additional patient specimens are taken at predetermined intervals during the test. The specimens are subjected to gene transcript analysis as
25 described above. In addition, the gene transcript changes noted in the earlier rat toxicity study are carefully evaluated as clinical markers in the followed patients. Changes in the gene transcript analyses are evaluated as indicators of toxicity by correlation with clinical signs
30 and symptoms and other laboratory results. In addition, subtraction is performed on individual patient specimens and on averaged patient specimens. The subtraction analysis highlights any toxicological changes in the treated patients. This is a highly refined determinant of
35 toxicity. The subtraction method also annotates clinical markers. Further subgroups can be analyzed by subtraction analysis, including, for example, 1) segregation by

occurrence and type of adverse effect; and 2) segregation by dosage.

6.12. GENE TRANSCRIPT IMAGING ANALYSIS IN CLINICAL STUDIES

A gene transcript imaging analysis (or multiple gene
5 transcript imaging analyses) is a useful tool in other
clinical studies. For example, the differences in gene
transcript imaging analyses before and after treatment can
be assessed for patients on placebo and drug treatment.
This method also effectively screens for clinical markers
10 to follow in clinical use of the drug.

6.13. COMPARATIVE GENE TRANSCRIPT ANALYSIS BETWEEN SPECIES

The subtraction method can be used to screen cDNA
libraries from diverse sources. For example, the same cell
types from different species can be compared by gene
15 transcript analysis to screen for specific differences,
such as in detoxification enzyme systems. Such testing
aids in the selection and validation of an animal model for
the commercial purpose of drug screening or toxicological
testing of drugs intended for human or animal use. When
20 the comparison between animals of different species is
shown in columns for each species, we refer to this as an
interspecies comparison, or zoo blot.

Embodiments of this invention may employ databases
such as those written using the FoxBASE programming
25 language commercially available from Microsoft Corporation.
Other embodiments of the invention employ other databases,
such as a random peptide database, a polymer database, a
synthetic oligomer database, or a oligonucleotide database
of the type described in U.S. Patent 5,270,170, issued
30 December 14, 1993 to Cull, et al., PCT International
Application Publication No. WO 9322684, published November
11, 1993, PCT International Application Publication No. WO
9306121, published April 1, 1993, or PCT International
Application Publication No. WO 9119818, published December
35 26, 1991. These four references (whose text is
incorporated herein by reference) include teaching which

may be applied in implementing such other embodiments of the present invention.

All references referred to in the preceding text are hereby expressly incorporated by reference herein.

- 5 Various modifications and variations of the described method and system of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific preferred
- 10 embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments.

TABLE 1

[illegible]

TABLE 2

Clone numbers 15000 through 20000

Libraries: HUVEC

Arranged by ABUNDANCE

Total clones analyzed: 5000

319 genes, for a total of 1713 Clones

	number	N	c	entry	s	descriptor
1	15365	67		HSRPL41		Riboptn L41
2	15004	65		NCY015004		INCYTE 015004
3	15638	63		NCY015638		INCYTE 015638
4	15390	50		NCY015390		INCYTE 015390
5	15193	47		HSFIB1		Fibronectin
6	15220	47		RRRPL9	R	Riboptn L9
7	15280	47		NCY015280		INCYTE 015280
8	15583	33		M62060		EST HHCH09 (IGR)
9	15662	31		HSACTCGR		Actin, gamma
10	15026	29		NCY015026		INCYTE 015026
11	15279	24		HSEF1AR		Elf 1-alpha
12	15027	23		NCY015027		INCYTE 015027
13	15033	20		NCY015033		INCYTE 015033
14	15198	20		NCY015198		INCYTE 015198
15	15809	20		HSCOLL1		Collagenase
16	15221	19		NCY015221		INCYTE 015221
17	15263	19		NCY015263		INCYTE 015263
18	15290	19		NCY015290		INCYTE 015290
19	15350	18		NCY015350		INCYTE 015350
20	15030	17		NCY015030		INCYTE 015030
21	15234	17		NCY015234		INCYTE 015234
22	15459	16		NCY015459		INCYTE 015459
23	15353	15		NCY015353		INCYTE 015353
24	15378	15		S76965		Ptn kinase inhib
25	15255	14		HUMTHYB4		Thymosin beta-4
26	15401	14		HSLIPCR		Lipocortin I
27	15425	14		HSPOLYAB		Poly-A bp
28	18212	14		HUMTHYMA		Thymosin, alpha
29	18216	14		HSMRP1		Motility relat ptn; MRP-1; CD-9
30	15189	13		HS18D		Interferon induc ptn 1-8D
31	15031	12		HUMFKBP		FK506 bp
32	15306	12		HSH2AZ		Histone H2A
33	15621	12		HUMLEC		Lectin, B-galbp, 14kDa
34	15789	11		NCY015789		INCYTE 015789
35	16578	11		HSRPS11		Riboptn S11
36	16632	11		M61984		EST HHCA13 (IGR)
37	18314	11		NCY018314		INCYTE 018314
38	15367	10		NCY015367		INCYTE 015367
39	15415	10		HSIFNIN1		interferon induc mRNA
40	15633	10		HSLDHAR		Lactate dehydrogenase
41	15813	10		CHKNMHCB		C Myosin heavy chain B
42	18210	10		NCY018210		INCYTE 018210
43	18233	10		HSRPII140		RNA polymerase II
44	18996	10		NCY018996		INCYTE 018996
45	15088	9		HUMFERL		Ferritin, light chain
46	15714	9		NCY015714		INCYTE 015714
47	15720	9		NCY015720		INCYTE 015720
48	15863	9		NCY015863		INCYTE 015863
49	16121	9		HSET		Endothelin
50	18252	9		NCY018252		INCYTE 018252
51	15351	8		HUMALBP		Lipid bp, adipocyte
52	15370	8		NCY015370		INCYTE 015370

TABLE 2 Con't

	number	N	c	entry	s	descriptor
53	15670	8		BTCIASHI	V	NADH-ubiq oxidoreductase
54	15795	8		NCY015795		INCYTE 015795
55	16245	8		NCY016245		INCYTE 016245
56	18262	8		NCY018262		INCYTE 018262
57	18321	8		HSRPL17		Riboptn L17
58	15126	7		XLRPL1BRF		Riboptn L1
59	15133	7		HSAC07		Actin, beta
60	15245	7		NCY015245		INCYTE 015245
61	15288	7		NCY015288		INCYTE 015288
62	15294	7		HSGAPDR		G-3-PD
63	15442	7		HUMLAMB		Laminin receptor, 54kDa
64	15485	7		HSNGMRNA		Uracil DNA glycosylase
65	16646	7		NCY016646		INCYTE 016646
66	18003	7		HUMPAIA		Plsmnogen activ gene
67	15032	6		HUMUB		Ubiquitin
68	15267	6		HSRPS8		Riboptn S8
69	15295	6		NCY015295		INCYTE 015295
70	15458	6		RNRPS10R	R	Riboptn S10
71	15832	6		RSGALEM	R	UDP-galactose epimerase
72	15928	6		HUMAPOJ		Apolipoptn J
73	16598	6		HUMTBMM40		Tubulin, beta
74	18218	6		NCY018218		INCYTE 018218
75	18499	6		HSP27		Hydrophobic ptn p27
76	18963	6		NCY018963		INCYTE 018963
77	18997	6		NCY018997		INCYTE 018997
78	15432	5		HSAGALAR		Galactosidase A, alpha
79	15475	5		NCY015475		INCYTE 015475
80	15721	5		NCY015721		INCYTE 015721
81	15865	5		NCY015865		INCYTE 015865
82	16270	5		NCY016270		INCYTE 016270
83	16886	5		NCY016886		INCYTE 016886
84	18500	5		NCY018500		INCYTE 018500
85	18503	5		NCY018503		INCYTE 018503
86	19672	5		RRRPL34	R	Riboptn L34
87	15086	4		XLRPL1AR	F	Riboptn L1a
88	15113	4		HUMIFNWRS		tRNA synthetase, trp
89	15242	4		NCY015242		INCYTE 015242
90	15249	4		NCY015249		INCYTE 015249
91	15377	4		NCY015377		INCYTE 015377
92	15407	4		NCY015407		INCYTE 015407
93	15473	4		NCY015473		INCYTE 015473
94	15588	4		HSRPS12		Riboptn S12
95	15684	4		HSEF1G		Elf 1-gamma
96	15782	4		NCY015782		INCYTE 015782
97	15916	4		HSRPS18		Riboptn S18
98	15930	4		NCY015930		INCYTE 015930
99	16108	4		NCY016108		INCYTE 016108
100	16133	4		NCY016133		INCYTE 016133

NORMAL MONONCYTE VS. ACTIVATED MACROPHAGE

Top 15 Most Abundant Genes

NORMAL

ACTIVATED

1	Elongation factor-1 alpha	Interleukin-1 beta
2	Ribosomal phosphoprotein	Macrophage inflammatory protein-1
3	Ribosomal protein S8 homolog	Interleukin-8
4	Beta-Globin	Lymphocyte activation gene
5	Ferritin H chain	Elongation factor-1 alpha
6	Ribosomal protein L7	Beta actin
7	Nucleoplasmin	Rantes T-cell specific protein
8	Ribosomal protein S20 homolog	Poly A binding protein
9	Transferrin receptor	Osteopontin; nephroponin
10	Poly-A binding protein	Tumor Necrosis Factor-alpha
11	Translationally controlled tumor ptn	INCYTE clone 011050
12	Ribosomal protein S25	Cu/Zn superoxide dismutase
13	Signal recognition particle SRP9	Adenylate cyclase (yeast homolog)
14	Histone H2A.Z	NGF-related B cell activation molecule
15	Ribosomal protein Ke-3	Protease Nexin-1, glial-derived

TABLE 3

TABLE 4

Libraries: THP-1
 Subtracting: HMC
 Sorted by ABUNDANCE
 Total clones analyzed: 7375

1057 genes, for a total of 2151 clones

number	entry	s descriptor	bgfreq	rfend	ratio
10022	HUMIL1	IL 1-beta	0	131	262.00
10036	HSMDNCF	IL-8	0	119	238.00
10089	HSLAG1CDN	Lymphocyte activ gene	0	71	142.00
10060	HUMTCSM	RANTES	0	23	46.000
10003	HUMMIP1A	MIP-1	3	121	40.333
10689	HSOP	Osteopontin	0	20	40.000
11050	NCY011050	INCYTE 011050	0	17	34.000
10937	HSTNFR	TNF-alpha	0	17	34.000
10176	HSSOD	Superoxide dismutase	0	14	28.000
10886	HSCDW40	B-cell activ,NGF-relat	0	10	20.000
10186	HUMAPR	Early resp PMA-induc	0	9	18.000
10967	HUMGDN	PN-1, glial-deriv	0	9	18.000
11353	NCY011353	INCYTE 011353	0	8	16.000
10298	NCY010298	INCYTE 010298	0	7	14.000
10215	HUM4COLA	Collagenase, type IV	0	6	12.000
10276	NCY010276	INCYTE 010276	0	6	12.000
10488	NCY010488	INCYTE 010488	0	6	12.000
11138	NCY011138	INCYTE 011138	0	6	12.000
10037	HUMCAPPRO	Adenylate cyclase	1	10	10.000
10840	HUMADCY	Adenylate cyclase	0	5	10.000
10672	HSCD44E	Cell adhesion glptn	0	5	10.000
12837	HUMCYCLOX	Cyclooxygenase-2	0	5	10.000
10001	NCY010001	INCYTE 010001	0	5	10.000
10005	NCY010005	INCYTE 010005	0	5	10.000
10294	NCY010294	INCYTE 010294	0	5	10.000
10297	NCY010297	INCYTE 010297	0	5	10.000
10403	NCY010403	INCYTE 010403	0	5	10.000
10699	NCY010699	INCYTE 010699	0	5	10.000
10966	NCY010966	INCYTE 010966	0	5	10.000
12092	NCY012092	INCYTE 012092	0	5	10.000
12549	HSRHOB	Oncogene rho	0	5	10.000
10691	HUMARF1BA	ADP-ribosylation fcctr	0	4	8.000
12106	HSADSS	Adenylosuccinate synthetase	0	4	8.000
10194	HSCATHL	Cathepsin L	0	4	8.000
10479	CLMCYCA	I Cyclin A	0	4	8.000
10031	NCY010031	INCYTE 010031	0	4	8.000
10203	NCY010203	INCYTE 010203	0	4	8.000
10288	NCY010288	INCYTE 010288	0	4	8.000
10372	NCY010372	INCYTE 010372	0	4	8.000
10471	NCY010471	INCYTE 010471	0	4	8.000
10484	NCY010484	INCYTE 010484	0	4	8.000
10859	NCY010859	INCYTE 010859	0	4	8.000
10890	NCY010890	INCYTE 010890	0	4	8.000
11511	NCY011511	INCYTE 011511	0	4	8.000
11868	NCY011868	INCYTE 011868	0	4	8.000
12820	NCY012820	INCYTE 012820	0	4	8.000
10133	HSI1RAP	IL-1 antagonist	0	4	8.000
10516	HUMP2A	Phosphatase, regul 2A	0	4	8.000
11063	HUMB94	TNF-induc response	0	4	8.000
11140	HSHB15RNA	HB15 gene; new Ig	0	3	6.000
10788	NCY001713	INCYTE 001713	0	3	6.000
10033	NCY010033	INCYTE 010033	0	3	6.000
10035	NCY010035	INCYTE 010035	0	3	6.000
10084	NCY010084	INCYTE 010084	0	3	6.000
10236	NCY010236	INCYTE 010236	0	3	6.000
10383	NCY010383	INCYTE 010383	0	3	6.000

TABLE 4 Con't

number	entry	s descriptor	bgfreq	rfend	ratio
10450	NCY010450	INCYTE 010450	0	3	6.000
10470	NCY010470	INCYTE 010470	0	3	6.000
10504	NCY010504	INCYTE 010504	0	3	6.000
10507	NCY010507	INCYTE 010507	0	3	6.000
10598	NCY010598	INCYTE 010598	0	3	6.000
10779	NCY010779	INCYTE 010779	0	3	6.000
10909	NCY010909	INCYTE 010909	0	3	6.000
10976	NCY010976	INCYTE 010976	0	3	6.000
10985	NCY010985	INCYTE 010985	0	3	6.000
11052	NCY011052	INCYTE 011052	0	3	6.000
11068	NCY011068	INCYTE 011068	0	3	6.000
11134	NCY011134	INCYTE 011134	0	3	6.000
11136	NCY011136	INCYTE 011136	0	3	6.000
11191	NCY011191	INCYTE 011191	0	3	6.000
11219	NCY011219	INCYTE 011219	0	3	6.000
11386	NCY011386	INCYTE 011386	0	3	6.000
11403	NCY011403	INCYTE 011403	0	3	6.000
11460	NCY011460	INCYTE 011460	0	3	6.000
11618	NCY011618	INCYTE 011618	0	3	6.000
11686	NCY011686	INCYTE 011686	0	3	6.000
12021	NCY012021	INCYTE 012021	0	3	6.000
12025	NCY012025	INCYTE 012025	0	3	6.000
12320	NCY012320	INCYTE 012320	0	3	6.000
12330	NCY012330	INCYTE 012330	0	3	6.000
12853	NCY012853	INCYTE 012853	0	3	6.000
14386	NCY014386	INCYTE 014386	0	3	6.000
14391	NCY014391	INCYTE 014391	0	3	6.000

TABLE 5

```

* Master menu for SUBTRACTION output
SET TALK OFF
SET SAFETY OFF
SET EXACT ON
SET TYPEAHEAD TO 0
CLEAR
SET DEVICE TO SCREEN
USE "SmartGuy:FoxBASE+/Mac:fox files:Clones.dbf"
GO TOP
STORE NUMBER TO INITIATE
GO BOTTOM
STORE NUMBER TO TERMINATE
STORE ' ' TO Target1
STORE ' ' TO Target2
STORE ' ' TO Target3
STORE ' ' TO Object1
STORE ' ' TO Object2
STORE ' ' TO Object3
STORE 0 TO ANAL
STORE 0 TO EMATCH
STORE 0 TO HMATCH
STORE 0 TO OMATCH
STORE 0 TO IMATCH
STORE 0 TO PTF
STORE 1 TO BAIL
DO WHILE .T.
  * Program.: Subtraction 2.fmt
  * Date.....: 10/11/94
  * Version.: FoxBASE+/Mac, revision 1.10
  * Notes.....: Format file Subtraction 2
  *
  SCREEN 1 TYPE 0 HEADING "Screen 1" AT 40,2 SIZE 286,492 PIXELS FONT "Geneva",9 COLOR 0,0,0,
  @ PIXELS 75,120 TO 178,241 STYLE 3871 COLOR 0,0,-1,24610,-1,8947
  @ PIXELS 27,134 SAY "Subtraction Menu" STYLE 65536 FONT "Geneva",274 COLOR 0,0,-1,-1,-1,-1
  @ PIXELS 117,126 GET EMATCH STYLE 65536 FONT "Chicago",12 PICTURE "e*c Exact " SIZE 15,62 CO
  @ PIXELS 135,126 GET HMATCH STYLE 65536 FONT "Chicago",12 PICTURE "e*c Homologous" SIZE 15,1
  @ PIXELS 153,126 GET OMATCH STYLE 65536 FONT "Chicago",12 PICTURE "e*c Other spc" SIZE 15,84
  @ PIXELS 90,152 SAY "Matches:" STYLE 65536 FONT "Geneva",12 COLOR 0,0,-1,-1,-1,-1
  @ PIXELS 171,126 GET Imatch STYLE 65536 FONT "Chicago",12 PICTURE "e*c Incyte" SIZE 15,63 CO
  @ PIXELS 252,137 GET initiate STYLE 0 FONT "Geneva",12 SIZE 15,70 COLOR 0,0,-1,-1,-1,-1
  @ PIXELS 252,236 GET terminate STYLE 0 FONT "Geneva",12 SIZE 15,70 COLOR 0,0,-1,-1,-1,-1
  @ PIXELS 252,35 SAY "Include clones:" STYLE 65536 FONT "Geneva",12 COLOR 0,0,-1,-1,-1,-1
  @ PIXELS 252,215 SAY "->" STYLE 65536 FONT "Geneva",14 COLOR 0,0,-1,-1,-1,-1
  @ PIXELS 198,126 GET PTF STYLE 65536 FONT "Chicago",12 PICTURE "e*c Print to file" SIZE 15,9
  @ PIXELS 90,9 TO 181,109 STYLE 3871 COLOR 0,0,-1,-25600,-1,-1
  @ PIXELS 90,288 TO 181,397 STYLE 3871 COLOR 0,0,-1,-25600,-1,-1
  @ PIXELS 81,296 SAY "Background:" STYLE 65536 FONT "Geneva",270 COLOR 0,0,-1,-1,-1,-1
  @ PIXELS 45,135 GET ANAL STYLE 65536 FONT "Chicago",12 PICTURE "e*R Overall;Function" SIZE 4
  @ PIXELS 81,26 SAY "Target:" STYLE 65536 FONT "Geneva",270 COLOR 0,0,-1,-1,-1,-1
  @ PIXELS 108,20 GET target1 STYLE 0 FONT "Geneva",9 SIZE 12,79 COLOR 0,0,-1,-1,-1,-1
  @ PIXELS 135,20 GET target2 STYLE 0 FONT "Geneva",9 SIZE 12,79 COLOR 0,0,-1,-1,-1,-1
  @ PIXELS 162,20 GET target3 STYLE 0 FONT "Geneva",9 SIZE 12,79 COLOR 0,0,-1,-1,-1,-1
  @ PIXELS 108,299 GET object1 STYLE 0 FONT "Geneva",9 SIZE 12,79 COLOR 0,0,-1,-1,-1,-1
  @ PIXELS 135,299 GET object2 STYLE 0 FONT "Geneva",9 SIZE 12,79 COLOR 0,0,-1,-1,-1,-1
  @ PIXELS 162,299 GET object3 STYLE 0 FONT "Geneva",9 SIZE 12,79 COLOR 0,0,-1,-1,-1,-1
  @ PIXELS 276,324 GET Bail STYLE 65536 FONT "Chicago",12 PICTURE "e*R Run;Bail out" SIZE 4112
  *
  * EOF: Subtraction.2.fmt
  READ
  IF Bail=2
    CLEAR
    CLOSE DATABASES
    USE "SmartGuy:FoxBASE+/Mac:fox files:clones.dbf"
    SET SAFETY ON
    SCREEN 1 OFF
    RETURN

```

```

ENDIF
STORE VAL(SYS(2)) TO STARTIME
STORE UPPER(Target1) TO Target1
STORE UPPER(Target2) TO Target2
STORE UPPER(Target3) TO Target3
STORE UPPER(Object1) TO Object1
STORE UPPER(Object2) TO Object2
STORE UPPER(Object3) TO Object3
clear
SET TALK ON
GAP = TERMINATE-INITIATE+1
GO INITIATE
COPY NEXT GAP FIELDS NUMBER,library,D,F,Z,R,ENTRY,S,DESCRIPTOR,START,RFEND,I TO TEMPNUM
USE TEMPNUM
COUNT TO TOT
COPY TO TEMPRED FOR D='E'.OR.D='O'.OR.D='H'.OR.D='N'.OR.D='I'
USE TEMPRED

IF Ematch=0 .AND. Hmatch=0 .AND. Omatch=0 .AND. IMATCH=0
COPY TO TEMPDESIG
ELSE
COPY STRUCTURE TO TEMPDESIG
USE TEMPDESIG
  IF Ematch=1
    APPEND FROM TEMPNUM FOR D='E'
  ENDIF
  IF Hmatch=1
    APPEND FROM TEMPNUM FOR D='H'
  ENDIF
  IF Omatch=1
    APPEND FROM TEMPNUM FOR D='O'
  ENDIF
  IF Imatch=1
    APPEND FROM TEMPNUM FOR D='I'.OR.D='X'
    *.OR.D='N'
  ENDIF
ENDIF
COUNT TO STARTOT

COPY STRUCTURE TO TEMPLIB
USE TEMPLIB
  APPEND FROM TEMPDESIG FOR library=UPPER(target1)
  IF target2<>'
    APPEND FROM TEMPDESIG FOR library=UPPER(target2)
  ENDIF
  IF target3<>'
    APPEND FROM TEMPDESIG FOR library=UPPER(target3)
  ENDIF
COUNT TO ANALTOT

USE TEMPDESIG
COPY STRUCTURE TO TEMPSUB
USE TEMPSUB
  APPEND FROM TEMPDESIG FOR library=UPPER(Object1)
  IF target2<>'
    APPEND FROM TEMPDESIG FOR library=UPPER(Object2)
  ENDIF
  IF target3<>'
    APPEND FROM TEMPDESIG FOR library=UPPER(Object3)
  ENDIF
COUNT TO SUBTRACTOT
SET TALK OFF
*****
* COMPRESSION SUBROUTINE A
? 'COMPRESSING QUERY LIBRARY'
USE TEMPLIB

```

```

SORT ON ENTRY, NUMBER TO LIBSORT
USE LIBSORT
COUNT TO IDGENE
REPLACE ALL RFEND WITH 1
MARK1 = 1
SW2=0
DO WHILE SW2=0 ROLL
  IF MARK1 >= IDGENE
    PACK
    COUNT TO AUNIQUE
    SW2=1
  LOOP
ENDIF
GO MARK1
DUP = 1
STORE ENTRY TO TESTA
STORE D TO DESIGA
SW = 0
DO WHILE SW=0 TEST
  SKIP
  STORE ENTRY TO TESTB
  STORE D TO DESIGB
  IF TESTA = TESTB.AND.DESIGA=DESIGB
    DELETE
    DUP = DUP+1
  LOOP
ENDIF
GO MARK1
REPLACE RFEND WITH DUP
MARK1 = MARK1+DUP
SW=1
LOOP
ENDDO TEST
LOOP
ENDDO ROLL
SORT ON RFEND/D, NUMBER TO TEMPTARSORT
USE TEMPTARSORT
*REPLACE ALL START WITH RFEND/IDGENE*10000
COUNT TO TEMPTARCO
*****
* COMPRESSION SUBROUTINE B
? 'COMPRESSING TARGET LIBRARY'
USE TEMPSUB
SORT ON ENTRY, NUMBER TO SUBSORT
USE SUBSORT
COUNT TO SUBGENE
REPLACE ALL RFEND WITH 1
MARK1 = 1
SW2=0
DO WHILE SW2=0 ROLL
  IF MARK1 >= SUBGENE
    PACK
    COUNT TO BUNIQUE
    SW2=1
  LOOP
ENDIF
GO MARK1
DUP = 1
STORE ENTRY TO TESTA
STORE D TO DESIGA
SW = 0
DO WHILE SW=0 TEST
  SKIP
  STORE ENTRY TO TESTB
  STORE D TO DESIGB
  IF TESTA = TESTB.AND.DESIGA=DESIGB

```

```

DELETE
DUP = DUP+1
LOOP
ENDIF
GO MARK1
REPLACE RFEND WITH DUP
MARK1 = MARK1+DUP
SW=1
LOOP
ENDDO TEST
LOOP
ENDDO ROLL
SORT ON RFEND/D,NUMBER TO TEMPSUBSORT
USE TEMPSUBSORT
*REPLACE ALL START WITH RFEND/IDGENE*10000
COUNT TO TEMPSUBCO
*****
*FUSION ROUTINE
? 'SUBTRACTING LIBRARIES'
USE SUBTRACTION
COPY STRUCTURE TO CRUNCHER
SELECT 2
USE TEMPSUBSORT
SELECT 1
USE CRUNCHER
APPEND FROM TEMPTARSORT
COUNT TO BAILOUT
MARK = 0

DO WHILE .T.
SELECT 1
MARK = MARK+1
IF MARK>BAILOUT
EXIT
ENDIF
GO MARK
STORE ENTRY TO SCANNER
SELECT 2
LOCATE FOR ENTRY=SCANNER
IF FOUND()
STORE RFEND TO BIT1
STORE RFEND TO BIT2
ELSE
STORE 1/2 TO BIT1
STORE 0 TO BIT2
ENDIF
SELECT 1
REPLACE BGFRSQ WITH BIT2
REPLACE ACTUAL WITH BIT1
LOOP
ENDDO

SELECT 1
REPLACE ALL RATIO WITH RFEND/ACTUAL
? 'DOING FINAL SORT BY RATIO'
SORT ON RATIO/D,BGFRSQ/D,DESCRIPTOR TO FINAL
USE FINAL
*****
set talk off
DO CASE
CASE PTF=0
SET DEVICE TO PRINT
SET PRINT ON
EJECT
CASE PTF=1
SET ALTERNATE TO "Adenoid.Patent.Figures.Subtraction.txt"

```

```
SET ALTERNATE ON
ENDCASE
```

```
STORE VAL(SYS(2)) TO FINTIME
IF FINTIME<STARTIME
STORE FINTIME+86400 TO FINTIME
ENDIF
STORE FINTIME - STARTIME TO COMPSEC
STORE COMPSEC/60 TO COMPMIN
```

```
*****
```

```
SET MARGIN TO 10
```

```
@1,1 SAY "Library Subtraction Analysis" STYLE 65536 FONT "Geneva",274 COLOR 0,0,0,-1,-1,-1
```

```
?
```

```
?
```

```
?
```

```
?
```

```
? date()
```

```
?? ' ' .
```

```
?? TIME()
```

```
? 'Clone numbers'
```

```
?? STR(INITIALS,5,0)
```

```
?? ' through'
```

```
?? STR(TERMINATE,6,0)
```

```
? 'Libraries:'
```

```
? Target1
```

```
IF Target2<>'
```

```
?? ' , '
```

```
?? Target2
```

```
ENDIF
```

```
IF Target3<>'
```

```
?? ' , '
```

```
?? Target3
```

```
ENDIF
```

```
? 'Subtracting:
```

```
? Object1
```

```
IF Object2<>'
```

```
?? ' , '
```

```
?? Object2
```

```
ENDIF
```

```
IF Object3<>'
```

```
?? ' , '
```

```
?? Object3
```

```
ENDIF
```

```
? 'Designations:'
```

```
IF Ematch=0 .AND. Hmatch=0 .AND. Omatch=0 .AND. Imatch=0
```

```
?? 'All'
```

```
ENDIF
```

```
IF Ematch=1
```

```
?? 'Exact,'
```

```
ENDIF
```

```
IF Hmatch=1
```

```
?? 'Human,'
```

```
ENDIF
```

```
IF Omatch=1
```

```
?? 'Other sp.'
```

```
ENDIF
```

```
IF Imatch=1
```

```
?? 'INCYTE'
```

```
ENDIF
```

```
IF ANAL=1
```

```
? 'Sorted by ABUNDANCE'
```

```
ENDIF
```

```
IF ANAL=2
```

```
? 'Arranged by FUNCTION'
```

```
ENDIF
```



```

? 'Total clones represented: '
?? STR(TOT,5,0)
? 'Total clones analyzed: '
?? STR(STARTOT,5,0)
? 'Total computation time: '
?? STR(COMPEN,5,2)
?? ' minutes'
?
? 'd = designation   f = distribution   z = location   r = function   s = species   i = inte
*****
SCREEN 1 TYPE 0 HEADING 'Screen 1' AT 40,2 SIZE 286,492 PIXELS FONT 'Geneva',9 COLOR 0,0,0,
DO CASE
CASE ANAL=1
?? STR(AUNIQUE,4,0)
?? ' genes, for a total of '
?? STR(ANAL/TOT,4,0)
?? ' clones'
?
SCREEN 1 TYPE 0 HEADING 'Screen 1' AT 40,2 SIZE 286,492 PIXELS FONT 'Geneva',7 COLOR 0,0,0,
list OFF fields number,D,F,Z,R,ENTRY,S,DESCRIPTOR,BGFREQ,RFEND,RATIO,I
SET PRINT OFF
CLOSE DATABASES
USE 'SmartGuy:FoxBASE+/Mac:fox files:clones.dbf'

CASE ANAL=2
* arrange/function
SET PRINT ON
SET HEADING ON
SCREEN 1 TYPE 0 HEADING 'Screen 1' AT 40,2 SIZE 286,492 PIXELS FONT 'Helvetica',268 COLOR 0
?
? '
? BINDING PROTEINS'
?
SCREEN 1 TYPE 0 HEADING 'Screen 1' AT 40,2 SIZE 286,492 PIXELS FONT 'Helvetica',265 COLOR 0
? 'Surface molecules and receptors:'
SCREEN 1 TYPE 0 HEADING 'Screen 1' AT 40,2 SIZE 286,492 PIXELS FONT 'Geneva',7 COLOR 0,0,0,
list OFF fields number,D,F,Z,R,ENTRY,S,DESCRIPTOR,BGFREQ,RFEND,RATIO,I FOR R='B'
?
SCREEN 1 TYPE 0 HEADING 'Screen 1' AT 40,2 SIZE 286,492 PIXELS FONT 'Helvetica',265 COLOR 0
? 'Calcium-binding proteins:'
SCREEN 1 TYPE 0 HEADING 'Screen 1' AT 40,2 SIZE 286,492 PIXELS FONT 'Geneva',7 COLOR 0,0,0,
list OFF fields number,D,F,Z,R,ENTRY,S,DESCRIPTOR,BGFREQ,RFEND,RATIO,I FOR R='C'
?
SCREEN 1 TYPE 0 HEADING 'Screen 1' AT 40,2 SIZE 286,492 PIXELS FONT 'Helvetica',265 COLOR 0
? 'Ligands and effectors:'
SCREEN 1 TYPE 0 HEADING 'Screen 1' AT 40,2 SIZE 286,492 PIXELS FONT 'Geneva',7 COLOR 0,0,0,
list OFF fields number,D,F,Z,R,ENTRY,S,DESCRIPTOR,BGFREQ,RFEND,RATIO,I FOR R='S'
?
SCREEN 1 TYPE 0 HEADING 'Screen 1' AT 40,2 SIZE 286,492 PIXELS FONT 'Helvetica',265 COLOR 0
? 'Other binding proteins:'
SCREEN 1 TYPE 0 HEADING 'Screen 1' AT 40,2 SIZE 286,492 PIXELS FONT 'Geneva',7 COLOR 0,0,0,
list OFF fields number,D,F,Z,R,ENTRY,S,DESCRIPTOR,BGFREQ,RFEND,RATIO,I FOR R='I'
?
SCREEN 1 TYPE 0 HEADING 'Screen 1' AT 40,2 SIZE 286,492 PIXELS FONT 'Helvetica',268 COLOR 0
? '
? ONCOGENES'
?
SCREEN 1 TYPE 0 HEADING 'Screen 1' AT 40,2 SIZE 286,492 PIXELS FONT 'Helvetica',265 COLOR 0
? 'General oncogenes:'
SCREEN 1 TYPE 0 HEADING 'Screen 1' AT 40,2 SIZE 286,492 PIXELS FONT 'Geneva',7 COLOR 0,0,0,
list OFF fields number,D,F,Z,R,ENTRY,S,DESCRIPTOR,BGFREQ,RFEND,RATIO,I FOR R='O'
?
SCREEN 1 TYPE 0 HEADING 'Screen 1' AT 40,2 SIZE 286,492 PIXELS FONT 'Helvetica',265 COLOR 0
? 'GTP-binding proteins:'
SCREEN 1 TYPE 0 HEADING 'Screen 1' AT 40,2 SIZE 286,492 PIXELS FONT 'Geneva',7 COLOR 0,0,0,
list OFF fields number,D,F,Z,R,ENTRY,S,DESCRIPTOR,BGFREQ,RFEND,RATIO,I FOR R='G'

```

SCREEN 1 TYPE 0 HEADING "Screen 1" AT 40,2 SIZE 286,492 PIXELS FONT "Helvetica",265 COLOR 0
 ? 'Viral elements:'
 SCREEN 1 TYPE 0 HEADING "Screen 1" AT 40,2 SIZE 286,492 PIXELS FONT "Geneva",7 COLOR 0,0,0,
 list OFF fields number,D,F,Z,R,ENTRY,S,DESCRIPTOR,BGFREQ,RFEND,RATIO,I FOR R='V'
 SCREEN 1 TYPE 0 HEADING "Screen 1" AT 40,2 SIZE 286,492 PIXELS FONT "Helvetica",265 COLOR 0
 ? 'Kinases and Phosphatases:'
 SCREEN 1 TYPE 0 HEADING "Screen 1" AT 40,2 SIZE 286,492 PIXELS FONT "Geneva",7 COLOR 0,0,0,
 list OFF fields number,D,F,Z,R,ENTRY,S,DESCRIPTOR,BGFREQ,RFEND,RATIO,I FOR R='Y'
 SCREEN 1 TYPE 0 HEADING "Screen 1" AT 40,2 SIZE 286,492 PIXELS FONT "Helvetica",265 COLOR 0
 ? 'Tumor-related antigens:'
 SCREEN 1 TYPE 0 HEADING "Screen 1" AT 40,2 SIZE 286,492 PIXELS FONT "Geneva",7 COLOR 0,0,0,
 list OFF fields number,D,F,Z,R,ENTRY,S,DESCRIPTOR,BGFREQ,RFEND,RATIO,I FOR R='A'
 ?
 SCREEN 1 TYPE 0 HEADING "Screen 1" AT 40,2 SIZE 286,492 PIXELS FONT "Helvetica",268 COLOR 0
 ? 'PROTEIN SYNTHETIC MACHINERY PROTEINS:'
 ?
 SCREEN 1 TYPE 0 HEADING "Screen 1" AT 40,2 SIZE 286,492 PIXELS FONT "Helvetica",265 COLOR 0
 ? 'Transcription and Nucleic Acid-binding proteins:'
 SCREEN 1 TYPE 0 HEADING "Screen 1" AT 40,2 SIZE 286,492 PIXELS FONT "Geneva",7 COLOR 0,0,0,
 list OFF fields number,D,F,Z,R,ENTRY,S,DESCRIPTOR,BGFREQ,RFEND,RATIO,I FOR R='D'
 SCREEN 1 TYPE 0 HEADING "Screen 1" AT 40,2 SIZE 286,492 PIXELS FONT "Helvetica",265 COLOR 0
 ? 'Translation:'
 SCREEN 1 TYPE 0 HEADING "Screen 1" AT 40,2 SIZE 286,492 PIXELS FONT "Geneva",7 COLOR 0,0,0,
 list OFF fields number,D,F,Z,R,ENTRY,S,DESCRIPTOR,BGFREQ,RFEND,RATIO,I FOR R='T'
 SCREEN 1 TYPE 0 HEADING "Screen 1" AT 40,2 SIZE 286,492 PIXELS FONT "Helvetica",265 COLOR 0
 ? 'Ribosomal proteins:'
 SCREEN 1 TYPE 0 HEADING "Screen 1" AT 40,2 SIZE 286,492 PIXELS FONT "Geneva",7 COLOR 0,0,0,
 list OFF fields number,D,F,Z,R,ENTRY,S,DESCRIPTOR,BGFREQ,RFEND,RATIO,I FOR R='R'
 SCREEN 1 TYPE 0 HEADING "Screen 1" AT 40,2 SIZE 286,492 PIXELS FONT "Helvetica",265 COLOR 0
 ? 'Protein processing:'
 SCREEN 1 TYPE 0 HEADING "Screen 1" AT 40,2 SIZE 286,492 PIXELS FONT "Geneva",7 COLOR 0,0,0,
 list OFF fields number,D,F,Z,R,ENTRY,S,DESCRIPTOR,BGFREQ,RFEND,RATIO,I FOR R='L'
 ?
 SCREEN 1 TYPE 0 HEADING "Screen 1" AT 40,2 SIZE 286,492 PIXELS FONT "Helvetica",268 COLOR 0
 ? 'ENZYMES:'
 ?
 SCREEN 1 TYPE 0 HEADING "Screen 1" AT 40,2 SIZE 286,492 PIXELS FONT "Helvetica",265 COLOR 0
 ? 'Ferrioproteins:'
 SCREEN 1 TYPE 0 HEADING "Screen 1" AT 40,2 SIZE 286,492 PIXELS FONT "Geneva",7 COLOR 0,0,0,
 list OFF fields number,D,F,Z,R,ENTRY,S,DESCRIPTOR,BGFREQ,RFEND,RATIO,I FOR R='F'
 SCREEN 1 TYPE 0 HEADING "Screen 1" AT 40,2 SIZE 286,492 PIXELS FONT "Helvetica",265 COLOR 0
 ? 'Proteases and inhibitors:'
 SCREEN 1 TYPE 0 HEADING "Screen 1" AT 40,2 SIZE 286,492 PIXELS FONT "Geneva",7 COLOR 0,0,0,
 list OFF fields number,D,F,Z,R,ENTRY,S,DESCRIPTOR,BGFREQ,RFEND,RATIO,I FOR R='P'
 SCREEN 1 TYPE 0 HEADING "Screen 1" AT 40,2 SIZE 286,492 PIXELS FONT "Helvetica",265 COLOR 0
 ? 'Oxidative phosphorylation:'
 SCREEN 1 TYPE 0 HEADING "Screen 1" AT 40,2 SIZE 286,492 PIXELS FONT "Geneva",7 COLOR 0,0,0,
 list OFF fields number,D,F,Z,R,ENTRY,S,DESCRIPTOR,BGFREQ,RFEND,RATIO,I FOR R='Z'
 SCREEN 1 TYPE 0 HEADING "Screen 1" AT 40,2 SIZE 286,492 PIXELS FONT "Helvetica",265 COLOR 0
 ? 'Sugar metabolism:'
 SCREEN 1 TYPE 0 HEADING "Screen 1" AT 40,2 SIZE 286,492 PIXELS FONT "Geneva",7 COLOR 0,0,0,
 list OFF fields number,D,F,Z,R,ENTRY,S,DESCRIPTOR,BGFREQ,RFEND,RATIO,I FOR R='Q'
 SCREEN 1 TYPE 0 HEADING "Screen 1" AT 40,2 SIZE 286,492 PIXELS FONT "Helvetica",265 COLOR 0
 ? 'Amino acid metabolism:'
 SCREEN 1 TYPE 0 HEADING "Screen 1" AT 40,2 SIZE 286,492 PIXELS FONT "Geneva",7 COLOR 0,0,0,

list OFF fields number,D,F,Z,R,ENTRY,S,DESCRIPTOR,BGFREQ,RFEND,RATIO,I FOR R='M'

SCREEN 1 TYPE 0 HEADING "Screen 1" AT 40,2 SIZE 286,492 PIXELS FONT "Helvetica",265 COLOR 0
 ? 'Nucleic acid metabolism:'

SCREEN 1 TYPE 0 HEADING "Screen 1" AT 40,2 SIZE 286,492 PIXELS FONT "Geneva",7 COLOR 0,0,0,
 list OFF fields number,D,F,Z,R,ENTRY,S,DESCRIPTOR,BGFREQ,RFEND,RATIO,I FOR R='N'

SCREEN 1 TYPE 0 HEADING "Screen 1" AT 40,2 SIZE 286,492 PIXELS FONT "Helvetica",265 COLOR 0
 ? 'Lipid metabolism:'

SCREEN 1 TYPE 0 HEADING "Screen 1" AT 40,2 SIZE 286,492 PIXELS FONT "Geneva",7 COLOR 0,0,0,
 list OFF fields number,D,F,Z,R,ENTRY,S,DESCRIPTOR,BGFREQ,RFEND,RATIO,I FOR R='W'

SCREEN 1 TYPE 0 HEADING "Screen 1" AT 40,2 SIZE 286,492 PIXELS FONT "Helvetica",265 COLOR 0
 ? 'Other enzymes:'

SCREEN 1 TYPE 0 HEADING "Screen 1" AT 40,2 SIZE 286,492 PIXELS FONT "Geneva",7 COLOR 0,0,0,
 list OFF fields number,D,F,Z,R,ENTRY,S,DESCRIPTOR,BGFREQ,RFEND,RATIO,I FOR R='E'

SCREEN 1 TYPE 0 HEADING "Screen 1" AT 40,2 SIZE 286,492 PIXELS FONT "Helvetica",265 COLOR 0
 ?
 ?
 ? MISCELLANEOUS CATEGORIES'
 ?

SCREEN 1 TYPE 0 HEADING "Screen 1" AT 40,2 SIZE 286,492 PIXELS FONT "Helvetica",265 COLOR 0
 ? 'Stress response:'

SCREEN 1 TYPE 0 HEADING "Screen 1" AT 40,2 SIZE 286,492 PIXELS FONT "Geneva",7 COLOR 0,0,0,
 list OFF fields number,D,F,Z,R,ENTRY,S,DESCRIPTOR,BGFREQ,RFEND,RATIO,I FOR R='H'

SCREEN 1 TYPE 0 HEADING "Screen 1" AT 40,2 SIZE 286,492 PIXELS FONT "Helvetica",265 COLOR 0
 ? 'Structural:'

SCREEN 1 TYPE 0 HEADING "Screen 1" AT 40,2 SIZE 286,492 PIXELS FONT "Geneva",7 COLOR 0,0,0,
 list OFF fields number,D,F,Z,R,ENTRY,S,DESCRIPTOR,BGFREQ,RFEND,RATIO,I FOR R='K'

SCREEN 1 TYPE 0 HEADING "Screen 1" AT 40,2 SIZE 286,492 PIXELS FONT "Helvetica",265 COLOR 0
 ? 'Other clones:'

SCREEN 1 TYPE 0 HEADING "Screen 1" AT 40,2 SIZE 286,492 PIXELS FONT "Geneva",7 COLOR 0,0,0,
 list OFF fields number,D,F,Z,R,ENTRY,S,DESCRIPTOR,BGFREQ,RFEND,RATIO,I FOR R='X'

SCREEN 1 TYPE 0 HEADING "Screen 1" AT 40,2 SIZE 286,492 PIXELS FONT "Helvetica",265 COLOR 0
 ? 'Clones of unknown function:'

SCREEN 1 TYPE 0 HEADING "Screen 1" AT 40,2 SIZE 286,492 PIXELS FONT "Geneva",7 COLOR 0,0,0,
 list OFF fields number,D,F,Z,R,ENTRY,S,DESCRIPTOR,BGFREQ,RFEND,RATIO,I FOR R='U'

ENDCASE

DO "Test print.prg"
 SET PRINT OFF
 SET DEVICE TO SCREEN
 CLOSE DATABASES
 ERASE TEMPLIB.DBF
 ERASE TEMPNUM.DBF
 ERASE TEMPDESIG.DBF
 SET MARGIN TO 0
 CLEAR
 LOOP
 ENDDO

```

*Northern (single), version 11-25-94
close databases
SET TALK OFF
SET PRINT OFF
SET EXACT OFF
CLEAR
STORE ' ' TO Eobject
STORE ' ' TO Dobject
STORE 0 TO Numb
STORE 0 TO Zog
STORE 1 TO Bail
DO WHILE .T.
* Program.: Northern (single).fmt
* Date....: 8/ 8/94
* Version.: FoxBASE+/Mac, revision 1.10
* Notes....: Format file Northern (single)
*
SCREEN 1 TYPE 0 HEADING "Screen 1" AT 40,2 SIZE 286,492 PIXELS FONT "Geneva",12 COLOR 0,0,0
@ PIXELS 15,81 TO 46,397 STYLE 28447 COLOR 0,0,-1,-25600,-1,-1
@ PIXELS 89,79 TO 192,422 STYLE 28447 COLOR 0,0,0,-25600,-1,-1
@ PIXELS 115,98 SAY "Entry #:" STYLE 65536 FONT "Geneva",12 COLOR 0,0,0,-1,-1,-1
@ PIXELS 115,173 GET Eobject STYLE 0 FONT "Geneva",12 SIZE 15,142 COLOR 0,0,0,-1,-1,-1
@ PIXELS 145,89 SAY "Description" STYLE 65536 FONT "Geneva",12 COLOR 0,0,0,-1,-1,-1
@ PIXELS 145,173 GET Dobject STYLE 0 FONT "Geneva",12 SIZE 15,241 COLOR 0,0,0,-1,-1,-1
@ PIXELS 35,89 SAY "Single Northern search screen" STYLE 65536 FONT "Geneva",12 SIZE 15,241 COLOR 0,0,0,-1,-1,-1
@ PIXELS 220,162 GET Bail STYLE 65536 FONT "Chicago",12 PICTURE "@*R Continue;Bail out" SIZE
@ PIXELS 175,98 SAY "Clone #:" STYLE 65536 FONT "Geneva",12 COLOR 0,0,0,-1,-1,-1
@ PIXELS 175,173 GET Numb STYLE 0 FONT "Geneva",12 SIZE 15,70 COLOR 0,0,0,-1,-1,-1
@ PIXELS 80,152 SAY "Enter any ONE of the following:" STYLE 65536 FONT "Geneva",12 COLOR -1,
*
* EOF: Northern (single).fmt
READ
IF Bail=2
CLEAR
screen 1 off
RETURN
ENDIF
USE "SmartGuy\FoxBASE+/Mac\Fox files\Lookup.dbf"
SET TALK ON

IF Eobject<>'
STORE UPPER(Eobject) to Eobject
SET SAFETY OFF
SORT ON Entry TO "Lookup entry.dbf"
SET SAFETY ON
USE "Lookup entry.dbf"
LOCATE FOR Look=Eobject
IF ..NOT.FOUND()
CLEAR
LOOP
ENDIF
BROWSE
STORE Entry TO Searchval
CLOSE DATABASES
ERASE "Lookup entry.dbf"
ENDIF

IF Dobject<>'
SET EXACT OFF
SET SAFETY OFF
SORT ON descriptor TO "Lookup descriptor.dbf"
SET SAFETY On
USE "Lookup descriptor.dbf"
LOCATE FOR UPPER(TRIM(descriptor))=UPPER(TRIM(Dobject))
IF ..NOT.FOUND()
CLEAR

```

```

LOOP
ENDIF
BROWSE
STORE Entry TO Searchval
CLOSE DATABASES
ERASE 'Lookup descriptor.dbf'
SET EXACT ON
ENDIF

IF Numb<>0
USE 'SmartGuy:FoxBASE+/Mac:Fox files:clones.dbf'
GO Numb
BROWSE
STORE Entry TO Searchval
ENDIF

CLEAR
? 'Northern analysis for entry '
?? Searchval
?
? 'Enter Y to proceed'
WAIT TO OK
CLEAR
IF UPPER(OK) <> 'Y'
screen 1 off
RETURN
ENDIF

* COMPRESSION SUBROUTINE FOR Library.dbf
? 'Compressing the Libraries file now...'
USE 'SmartGuy:FoxBASE+/Mac:Fox files:libraries.dbf'
SET SAFETY OFF
SORT ON library TO 'Compressed libraries.dbf'
* FOR entered>0
SET SAFETY ON
USE 'Compressed libraries.dbf'
DELETE FOR entered=0
PACK
COUNT TO TOT
MARK1 = 1
SW2=0
DO WHILE SW2=0 ROLL
  IF MARK1 >= TOT
    PACK
    SW2=1
    LOOP
  ENDIF
  GO MARK1
  STORE library TO TESTA
  SKIP
  STORE Library TO TESTB
  IF TESTA = TESTB
    DELETE
  ENDIF
  MARK1 = MARK1+1
  LOOP
ENDDO ROLL

* Northern analysis
CLEAR
? 'Doing the northern now...'
SET TALK ON
USE 'SmartGuy:FoxBASE+/Mac:Fox files:clones.dbf'
SET SAFETY OFF
COPY TO 'Hits.dbf' FOR entry=searchval
SET SAFETY ON

```

```

* MASTER ANALYSIS 3; VERSION 12-9-94
* Master menu for analysis output
CLOSE DATABASES
SET TALK OFF
SET SAFETY OFF
CLEAR
SET DEVICE TO SCREEN
SET DEFAULT TO "SmartGuy:FoxBASE+/Mac:fox files:Output programs:"
USE "SmartGuy:FoxBASE+/Mac:fox files:Clones.dbf"
GO TOP
STORE NUMBER TO INITIATE
GO BOTTOM
STORE NUMBER TO TERMINATE
STORE 0 TO ENTIRE
STORE 0 TO CONDEN
STORE 0 TO ANAL
STORE 0 TO EMATCH
STORE 0 TO HMATCH
STORE 0 TO OMATCH
STORE 0 TO IMATCH
STORE 0 TO XMATCH
STORE 0 TO PRINTON
STORE 0 TO PTF
DO WHILE .T.
* Program.: Master analysis.fmt
* Date.....: 12/ 9/94
* Version.: FoxBASE+/Mac, revision 1.10
* Notes.....: Format file Master analysis
*
SCREEN 1 TYPE 0 HEADING "Screen 1" AT 40,2 SIZE 286,492 PIXELS FONT "Geneva",9 COLOR 0,0,0,
@ PIXELS 39,255 TO 277,430 STYLE 28447 COLOR 0,0,-1,-25600,-1,-1
@ PIXELS 75,120 TO 178,241 STYLE 3871 COLOR 0,0,-1,-25600,-1,-1
@ PIXELS 27,98 SAY "Customized Output Menu" STYLE 65536 FONT "Geneva",274 COLOR 0,0,-1,-1,-1,-1
@ PIXELS 45,54 GET conden STYLE 65536 FONT "Chicago",12 PICTURE "@*C Condensed format" SIZE
@ PIXELS 54,261 GET anal STYLE 65536 FONT "Chicago",12 PICTURE "@*RV Sort/number;Sort/entry;"
@ PIXELS 117,126 GET EMATCH STYLE 65536 FONT "Chicago",12 PICTURE "@*C Exact " SIZE 15,62 CO
@ PIXELS 135,126 GET HMATCH STYLE 65536 FONT "Chicago",12 PICTURE "@*C Homologous" SIZE 15,1
@ PIXELS 153,126 GET OMATCH STYLE 65536 FONT "Chicago",12 PICTURE "@*C Other spc" SIZE 15,84
@ PIXELS 90,152 SAY "Matches:" STYLE 65536 FONT "Geneva",268 COLOR 0,0,-1,-1,-1,-1
@ PIXELS 63,54 GET PRINTON STYLE 65536 FONT "Chicago",12 PICTURE "@*C Include clone listing"
@ PIXELS 171,126 GET Imatch STYLE 65536 FONT "Chicago",12 PICTURE "@*C Incyte" SIZE 15,65 CO
@ PIXELS 252,146 GET initiate STYLE 0 FONT "Geneva",12 SIZE 15,70 COLOR 0,0,-1,-1,-1,-1
@ PIXELS 270,146 GET terminate STYLE 0 FONT "Geneva",12 SIZE 15,70 COLOR 0,0,-1,-1,-1,-1
@ PIXELS 234,134 SAY "Include clones " STYLE 65536 FONT "Geneva",12 COLOR 0,0,-1,-1,-1,-1
@ PIXELS 270,125 SAY "->" STYLE 65536 FONT "Geneva",14 COLOR 0,0,-1,-1,-1,-1
@ PIXELS 198,126 GET PTF STYLE 65536 FONT "Chicago",12 PICTURE "@*C Print to file" SIZE 15,9
@ PIXELS 189,0 TO 257,120 STYLE 3871 COLOR 0,0,-1,-25600,-1,-1
@ PIXELS 209,8 SAY "Library selection" STYLE 65536 FONT "Geneva",266 COLOR 0,0,-1,-1,-1,-1
@ PIXELS 227,18 GET ENTIRE STYLE 65536 FONT "Chicago",12 PICTURE "@*RV All;Selected" SIZE 16
*
* EOF: Master analysis.fmt
READ
IF ANAL=9
CLEAR
CLOSE DATABASES
ERASE TEMPMASER.DBF
USE "SmartGuy:FoxBASE+/Mac:fox files:clones.dbf"
SET SAFETY ON
SCREEN 1 OFF
RETURN
ENDIF
clear
? INITIATE
? TERMINATE
? CONDEN
? ANAL

```



```

? ematch
? Hmatch
? Omatch
? IMATCH
SET TALK ON
IF ENTIRE=2
USE "Unique libraries.dbf"
REPLACE ALL i WITH ' '
BROWSE FIELDS i, libname, library, total, entered AT 0,0
ENDIF
USE "SmartGuy:FoxBASE+/Mac:fox files:clones.dbf"
*COPY TO TEMPNUM FOR NUMBER>=INITIATE.AND.NUMBER<=TERMINATE
*USE TEMPNUM
COPY STRUCTURE TO TEMPLIB
USE TEMPLIB
IF ENTIRE=1
APPEND FROM "SmartGuy:FoxBASE+/Mac:fox files:Clones.dbf"
ENDIF
IF ENTIRE=2
USE "Unique libraries.dbf"
COPY TO SELECTED FOR UPPER(i)='Y'
USE SELECTED
STORE RECCOUNT() TO STOPIT
MARK=1
DO WHILE .T.
IF MARK>STOPIT
CLEAR
EXIT
ENDIF
USE SELECTED
GO MARK
STORE library TO THISONE
? 'COPYING '
?? THISONE
USE TEMPLIB
APPEND FROM "SmartGuy:FoxBASE+/Mac:fox files:Clones.dbf" FOR library=THISONE
STORE MARK+1 TO MARK
LOOP
ENDDO
ENDIF
USE "SmartGuy:FoxBASE+/Mac:fox files:clones.dbf"
COUNT TO STARTOT
COPY STRUCTURE TO TEMPDESIG
USE TEMPDESIG
IF Ematch=0 .AND. Hmatch=0 .AND. Omatch=0 .AND. IMATCH=0
APPEND FROM TEMPLIB
ENDIF
IF Ematch=1
APPEND FROM TEMPLIB FOR D='E'
ENDIF
IF Hmatch=1
APPEND FROM TEMPLIB FOR D='H'
ENDIF
IF Omatch=1
APPEND FROM TEMPLIB FOR D='O'
ENDIF
IF Imatch=1
APPEND FROM TEMPLIB FOR D='I'.OR.D='X'.OR.D='N'
ENDIF
IF Xmatch=1
APPEND FROM TEMPLIB FOR D='X'
ENDIF
COUNT TO ANALTOT
set talk off
*****
DO CASE

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```

CASE PTF=0
SET DEVICE TO PRINT
SET PRINT ON
EJECT
CASE PTF=1
SET ALTERNATE TO "Total function sort.txt"
*SET ALTERNATE TO "H and O function sort.txt"
*SET ALTERNATE TO "Shear Stress HUVEC 2:Abundance sort.txt"
*SET ALTERNATE TO "Shear Stress HUVEC 2:Abundance con.txt"
*SET ALTERNATE TO "Shear Stress HUVEC 2:Function sort.txt"
*SET ALTERNATE TO "Shear Stress HUVEC 2:Distribution sort.txt"
*SET ALTERNATE TO "Shear stress HUVEC 1:Clone list.txt"
*SET ALTERNATE TO "Shear Stress HUVEC 2:Location sort.txt"
SET ALTERNATE ON
ENDCASE
*****
IF PRINION=1
@1,30 SAY "Database Subset Analysis" STYLE 65536 FONT "Geneva",274 COLOR 0,0,0,-1,-1,-1
ENDIF
?
?
?
?
? date()
?? '
?? TIME()
? 'Clone numbers '
?? STR(INITIATE,6,0)
?? ' through '
?? STR(TERMINATE,6,0)
? 'Libraries: '
IF ENTIRE=1
? 'All libraries'
ENDIF
IF ENTIRE=2
MARK=1
DO WHILE .T.
IF MARK>STOPIT
EXIT
ENDIF
USE SELECTED
GO MARK
? ' '
?? TRIM(libname)
STORE MARK+1 TO MARK
LOOP
ENDDO
ENDIF
? 'Designations: '
IF Ematch=0 .AND. Hmatch=0 .AND. Omatch=0 .AND. Imatch=0
?? 'All'
ENDIF
IF Ematch=1
?? 'Exact,'
ENDIF
IF Hmatch=1
?? 'Human,'
ENDIF
IF Omatch=1
?? 'Other sp.'
ENDIF
IF Imatch=1
?? 'INCYTE'
ENDIF
IF Xmatch=1
?? 'EST'

```

```

ENDIF
IF CONDEN=1
? 'Condensed format analysis'
ENDIF
IF ANAL=1
? 'Sorted by NUMBER'
ENDIF
IF ANAL=2
? 'Sorted by ENTRY'
ENDIF
IF ANAL=3
? 'Arranged by ABUNDANCE'
ENDIF
IF ANAL=4
? 'Sorted by INTEREST'
ENDIF
IF ANAL=5
? 'Arranged by LOCATION'
ENDIF
IF ANAL=6
? 'Arranged by DISTRIBUTION'
ENDIF
IF ANAL=7
? 'Arranged by FUNCTION'
ENDIF
? 'Total clones represented: '
?? STR(STARTOT,6,0)
? 'Total clones analyzed: '
?? STR(ANALTOT,6,0)
?
? 'l = library    d = designation    f = distribution    z = location    r = function    c = cer
?
*****
USE TEMPDESIG
SCREEN 1 TYPE 0 HEADING "Screen 1" AT 40,2 SIZE 286,492 PIXELS FONT "Geneva",7 COLOR 0,0,0,
DO CASE
CASE ANAL=1
* sort/number
SET HEADING ON
IF CONDEN=1
SORT TO TEMP1 ON ENTRY,NUMBER
DO "COMPRESSION number.PRG"
ELSE
SORT TO TEMP1 ON NUMBER
USE TEMP1
list off fields number,L,D,F,Z,R,C,ENTRY,S,DESCRIPTOR
*list off fields number,L,D,F,Z,R,C,ENTRY,S,DESCRIPTOR,LENGTH,RFEND,INIT,I
CLOSE DATABASES
ERASE TEMP1.DBF
ENDIF

CASE ANAL=2
* sort/DESCRIPTOR
SET HEADING ON
*SORT TO TEMP1 ON DESCRIPTOR,ENTRY,NUMBER/S for D='E'.OR.D='H'.OR.D='O'.OR.D='X'.OR.D='I'
*SORT TO TEMP1 ON ENTRY,DESCRIPTOR,NUMBER/S for D='E'.OR.D='H'.OR.D='O'.OR.D='X'.OR.D='I'
SORT TO TEMP1 ON ENTRY,START/S for D='E'.OR.D='H'.OR.D='O'.OR.D='X'.OR.D='I'
IF CONDEN=1
DO "COMPRESSION entry.PRG"
ELSE
USE TEMP1
list off fields number,L,D,F,Z,R,C,ENTRY,S,DESCRIPTOR,LENGTH,RFEND,INIT,I
CLOSE DATABASES
ERASE TEMP1.DBF
ENDIF

```

```

CASE ANAL=3
* sort by abundance
SET HEADING ON
SORT TO TEMP1 ON ENTRY,NUMBER for D='E'.OR.D='H'.OR.D='O'.OR.D='X'.OR.D='I'
DO "COMPRESSION abundance.PRG"

CASE ANAL=4
* sort/interest
SET HEADING ON
IF CONDEN=1
SORT TO TEMP1 ON ENTRY,NUMBER FOR I>0
DO "COMPRESSION interest.PRG"
ELSE
SORT ON I/D,ENTRY TO TEMP1 FOR I>1
USE TEMP1
list off fields number,L,D,F,Z,R,C,ENTRY,S,DESCRIPTOR,LENGTH,RFEND,INIT,I
CLOSE DATABASES
ERASE TEMP1.DBF
ENDIF

CASE ANAL=5
* arrange/location
SET HEADING ON
STORE 4 TO AMPLIFIER
? 'Nuclear:'
SORT ON ENTRY,NUMBER FIELDS RFEND,NUMBER,L,D,F,Z,R,C,ENTRY,S,DESCRIPTOR,LENGTH,INIT,I,COMMEN
IF CONDEN=1
DO "Compression location.prg"
ELSE
DO "Normal subroutine 1"
ENDIF
? 'Cytoplasmic:'
SORT ON ENTRY,NUMBER FIELDS RFEND,NUMBER,L,D,F,Z,R,C,ENTRY,S,DESCRIPTOR,LENGTH,INIT,I,COMMEN
IF CONDEN=1
DO "Compression location.prg"
ELSE
DO "Normal subroutine 1"
ENDIF
? 'Cytoskeleton:'
SORT ON ENTRY,NUMBER FIELDS RFEND,NUMBER,L,D,F,Z,R,C,ENTRY,S,DESCRIPTOR,LENGTH,INIT,I,COMMEN
IF CONDEN=1
DO "Compression location.prg"
ELSE
DO "Normal subroutine 1"
ENDIF
? 'Cell surface:'
SORT ON ENTRY,NUMBER FIELDS RFEND,NUMBER,L,D,F,Z,R,C,ENTRY,S,DESCRIPTOR,LENGTH,INIT,I,COMMEN
IF CONDEN=1
DO "Compression location.prg"
ELSE
DO "Normal subroutine 1"
ENDIF
? 'Intracellular membrane:'
SORT ON ENTRY,NUMBER FIELDS RFEND,NUMBER,L,D,F,Z,R,C,ENTRY,S,DESCRIPTOR,LENGTH,INIT,I,COMMEN
IF CONDEN=1
DO "Compression location.prg"
ELSE
DO "Normal subroutine 1"
ENDIF
? 'Mitochondrial:'
SORT ON ENTRY,NUMBER FIELDS RFEND,NUMBER,L,D,F,Z,R,C,ENTRY,S,DESCRIPTOR,LENGTH,INIT,I,COMMEN
IF CONDEN=1
DO "Compression location.prg"
ELSE
DO "Normal subroutine 1"
ENDIF

```

```

? 'Secreted:'
SORT ON ENTRY,NUMBER FIELDS RFEND,NUMBER,L,D,F,Z,R,C,ENTRY,S,DESCRIPTOR,LENGTH,INIT,I,COMMEN
IF CONDEN=1
DO "Compression location.prg"
ELSE
DO "Normal subroutine 1"
ENDIF
? 'Other:'
SORT ON ENTRY,NUMBER FIELDS RFEND,NUMBER,L,D,F,Z,R,C,ENTRY,S,DESCRIPTOR,LENGTH,INIT,I,COMMEN
IF CONDEN=1
DO "Compression location.prg"
ELSE
DO "Normal subroutine 1"
ENDIF
? 'Unknown:'
SORT ON ENTRY,NUMBER FIELDS RFEND,NUMBER,L,D,F,Z,R,C,ENTRY,S,DESCRIPTOR,LENGTH,INIT,I,COMMEN
IF CONDEN=1
DO "Compression location.prg"
ELSE
DO "Normal subroutine 1"
ENDIF
IF CONDEN=1
SET DEVICE TO PRINTER
SET PRINTER ON
EJECT
DO "Output heading.prg"
USE "Analysis location.dbf"
DO "Create bargraph.prg"
SET HEADING OFF
? '          FUNCTIONAL CLASS                TOTAL    UNIQUE    NEW    % TOTAL'
?
LIST OFF FIELDS Z,NAME,CLONES,GENES,NEW,PERCENT,GRAPH
CLOSE DATABASES
ERASE TEMP2.DBF
SET HEADING ON
*USE "SmartGuy:FoxBASE+/Mac:fox files:TEMPMASTER.dbf"
ENDIF

CASE ANAL=6
* arrange/distribution
SET HEADING ON
STORE 3 TO AMPLIFIER
? 'Cell/tissue specific distribution:'
SORT ON ENTRY,NUMBER FIELDS RFEND,NUMBER,L,D,F,Z,R,C,ENTRY,S,DESCRIPTOR,LENGTH,INIT,I,COMMEN
IF CONDEN=1
DO "Compression distrib.prg"
ELSE
DO "Normal subroutine 1"
ENDIF
? 'Non-specific distribution:'
SORT ON ENTRY,NUMBER FIELDS RFEND,NUMBER,L,D,F,Z,R,C,ENTRY,S,DESCRIPTOR,LENGTH,INIT,I,COMMEN
IF CONDEN=1
DO "Compression distrib.prg"
ELSE
DO "Normal subroutine 1"
ENDIF
? 'Unknown distribution:'
SORT ON ENTRY,NUMBER FIELDS RFEND,NUMBER,L,D,F,Z,R,C,ENTRY,S,DESCRIPTOR,LENGTH,INIT,I,COMMEN
IF CONDEN=1
DO "Compression distrib.prg"
ELSE
DO "Normal subroutine 1"
ENDIF
IF CONDEN=1
SET DEVICE TO PRINTER
SET PRINTER ON

```

```

EJECT
DO "Output heading.prg"
USE "Analysis distribution.dbf"
DO "Create bargraph.prg"
SET HEADING OFF
? '          FUNCTIONAL CLASS          TOTAL  UNIQUE  % TOTAL'
?
LIST OFF FIELDS P.NAME,CLONES,GENES,PERCENT,GRAPH
CLOSE DATABASES
ERASE TEMP2.DBF
SET HEADING ON
*USE "SmartGuy:FoxBASE+/Mac:fox files:TEMPMASTER.dbf"
ENDIF

CASE ANAL=7
* arrange/function
SET HEADING ON
STORE 10 TO AMPLIFIER
? '          BINDING PROTEINS'
?
? 'Surface molecules and receptors:'
SORT ON ENTRY,NUMBER FIELDS RFEND,NUMBER,L,D,F,Z,R,C,ENTRY,S,DESCRIPTOR,LENGTH,INIT,I,COMMEN
IF CONDEN=1
DO "Compression function.prg"
ELSE
DO "Normal subroutine 1"
ENDIF
? 'Calcium-binding proteins:'
SORT ON ENTRY,NUMBER FIELDS RFEND,NUMBER,L,D,F,Z,R,C,ENTRY,S,DESCRIPTOR,LENGTH,INIT,I,COMMEN
IF CONDEN=1
DO "Compression function.prg"
ELSE
DO "Normal subroutine 1"
ENDIF
? 'Ligands and effectors:'
SORT ON ENTRY,NUMBER FIELDS RFEND,NUMBER,L,D,F,Z,R,C,ENTRY,S,DESCRIPTOR,LENGTH,INIT,I,COMMEN
IF CONDEN=1
DO "Compression function.prg"
ELSE
DO "Normal subroutine 1"
ENDIF
? 'Other binding proteins:'
SORT ON ENTRY,NUMBER FIELDS RFEND,NUMBER,L,D,F,Z,R,C,ENTRY,S,DESCRIPTOR,LENGTH,INIT,I,COMMEN
IF CONDEN=1
DO "Compression function.prg"
ELSE
DO "Normal subroutine 1"
ENDIF
*EJECT
? '          ONCOGENES'
?
? 'General oncogenes:'
SORT ON ENTRY,NUMBER FIELDS RFEND,NUMBER,L,D,F,Z,R,C,ENTRY,S,DESCRIPTOR,LENGTH,INIT,I,COMMEN
IF CONDEN=1
DO "Compression function.prg"
ELSE
DO "Normal subroutine 1"
ENDIF
? 'GTP-binding proteins:'
SORT ON ENTRY,NUMBER FIELDS RFEND,NUMBER,L,D,F,Z,R,C,ENTRY,S,DESCRIPTOR,LENGTH,INIT,I,COMMEN
IF CONDEN=1
DO "Compression function.prg"
ELSE
DO "Normal subroutine 1"
ENDIF
? 'Viral elements:'

```



```

SORT ON ENTRY,NUMBER FIELDS RFEND,NUMBER,L,D,F,Z,R,C,ENTRY,S,DESCRIPTOR,LENGTH,INIT,I,COMMEN
IF CONDEN=1
DO "Compression function.prg"
ELSE
DO "Normal subroutine 1"
ENDIF
? 'Kinases and Phosphatases:'
SORT ON ENTRY,NUMBER FIELDS RFEND,NUMBER,L,D,F,Z,R,C,ENTRY,S,DESCRIPTOR,LENGTH,INIT,I,COMMEN
IF CONDEN=1
DO "Compression function.prg"
ELSE
DO "Normal subroutine 1"
ENDIF
? 'Tumor-related antigens:'
SORT ON ENTRY,NUMBER FIELDS RFEND,NUMBER,L,D,F,Z,R,C,ENTRY,S,DESCRIPTOR,LENGTH,INIT,I,COMMEN
IF CONDEN=1
DO "Compression function.prg"
ELSE
DO "Normal subroutine 1"
ENDIF
*EJECT
? '
                                PROTEIN SYNTHETIC MACHINERY PROTEINS'
?
? 'Transcription and Nucleic Acid-binding proteins:'
SORT ON ENTRY,NUMBER FIELDS RFEND,NUMBER,L,D,F,Z,R,C,ENTRY,S,DESCRIPTOR,LENGTH,INIT,I,COMMEN
IF CONDEN=1
DO "Compression function.prg"
ELSE
DO "Normal subroutine 1"
ENDIF
? 'Translation:'
SORT ON ENTRY,NUMBER FIELDS RFEND,NUMBER,L,D,F,Z,R,C,ENTRY,S,DESCRIPTOR,LENGTH,INIT,I,COMMEN
IF CONDEN=1
DO "Compression function.prg"
ELSE
DO "Normal subroutine 1"
ENDIF
? 'Ribosomal proteins:'
SORT ON ENTRY,NUMBER FIELDS RFEND,NUMBER,L,D,F,Z,R,C,ENTRY,S,DESCRIPTOR,LENGTH,INIT,I,COMMEN
IF CONDEN=1
DO "Compression function.prg"
ELSE
DO "Normal subroutine 1"
ENDIF
? 'Protein processing:'
SORT ON ENTRY,NUMBER FIELDS RFEND,NUMBER,L,D,F,Z,R,C,ENTRY,S,DESCRIPTOR,LENGTH,INIT,I,COMMEN
IF CONDEN=1
DO "Compression function.prg"
ELSE
DO "Normal subroutine 1"
ENDIF
*EJECT
? '
                                ENZYMES'
?
? 'Ferroteins:'
SORT ON ENTRY,NUMBER FIELDS RFEND,NUMBER,L,D,F,Z,R,C,ENTRY,S,DESCRIPTOR,LENGTH,INIT,I,COMMEN
IF CONDEN=1
DO "Compression function.prg"
ELSE
DO "Normal subroutine 1"
ENDIF
? 'Proteases and inhibitors:'
SORT ON ENTRY,NUMBER FIELDS RFEND,NUMBER,L,D,F,Z,R,C,ENTRY,S,DESCRIPTOR,LENGTH,INIT,I,COMMEN
IF CONDEN=1
DO "Compression function.prg"
ELSE

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```

DO "Normal subroutine 1"
ENDIF
? 'Oxidative phosphorylation:'
SORT ON ENTRY,NUMBER FIELDS RFEND,NUMBER,L,D,F,Z,R,C,ENTRY,S,DESCRIPTOR,LENGTH,INIT,I,COMMEN
IF CONDEN=1
DO "Compression function.prg"
ELSE
DO "Normal subroutine 1"
ENDIF
? 'Sugar metabolism:'
SORT ON ENTRY,NUMBER FIELDS RFEND,NUMBER,L,D,F,Z,R,C,ENTRY,S,DESCRIPTOR,LENGTH,INIT,I,COMMEN
IF CONDEN=1
DO "Compression function.prg"
ELSE
DO "Normal subroutine 1"
ENDIF
? 'Amino acid metabolism:'
SORT ON ENTRY,NUMBER FIELDS RFEND,NUMBER,L,D,F,Z,R,C,ENTRY,S,DESCRIPTOR,LENGTH,INIT,I,COMMEN
IF CONDEN=1
DO "Compression function.prg"
ELSE
DO "Normal subroutine 1"
ENDIF
? 'Nucleic acid metabolism:'
SORT ON ENTRY,NUMBER FIELDS RFEND,NUMBER,L,D,F,Z,R,C,ENTRY,S,DESCRIPTOR,LENGTH,INIT,I,COMMEN
IF CONDEN=1
DO "Compression function.prg"
ELSE
DO "Normal subroutine 1"
ENDIF
? 'Lipid metabolism:'
SORT ON ENTRY,NUMBER FIELDS RFEND,NUMBER,L,D,F,Z,R,C,ENTRY,S,DESCRIPTOR,LENGTH,INIT,I,COMMEN
IF CONDEN=1
DO "Compression function.prg"
ELSE
DO "Normal subroutine 1"
ENDIF
? 'Other enzymes:'
SORT ON ENTRY,NUMBER FIELDS RFEND,NUMBER,L,D,F,Z,R,C,ENTRY,S,DESCRIPTOR,LENGTH,INIT,I,COMMEN
IF CONDEN=1
DO "Compression function.prg"
ELSE
DO "Normal subroutine 1"
ENDIF
*EJECT
? '
?
? 'Stress response:'
SORT ON ENTRY,NUMBER FIELDS RFEND,NUMBER,L,D,F,Z,R,C,ENTRY,S,DESCRIPTOR,LENGTH,INIT,I,COMMEN
IF CONDEN=1
DO "Compression function.prg"
ELSE
DO "Normal subroutine 1"
ENDIF
? 'Structural:'
SORT ON ENTRY,NUMBER FIELDS RFEND,NUMBER,L,D,F,Z,R,C,ENTRY,S,DESCRIPTOR,LENGTH,INIT,I,COMMEN
IF CONDEN=1
DO "Compression function.prg"
ELSE
DO "Normal subroutine 1"
ENDIF
? 'Other clones:'
SORT ON ENTRY,NUMBER FIELDS RFEND,NUMBER,L,D,F,Z,R,C,ENTRY,S,DESCRIPTOR,LENGTH,INIT,I,COMMEN
IF CONDEN=1
DO "Compression function.prg"
ELSE

```

MISCELLANEOUS CATEGORIES'

```

DO "Normal subroutine 1"
ENDIF
? "Clones of unknown function:"
SORT ON ENTRY,NUMBER FIELDS RFEND,NUMBER,L,D,F,Z,R,C,ENTRY,S,DESCRIPTOR,LENGTH,INIT,I,COMMEN
IF CONDEN=1
DO "Compression function.prg"
ELSE
DO "Normal subroutine 1"
ENDIF

IF CONDEN=1
EJECT
*SET DEVICE TO PRINTER
*SET PRINT ON
DO "Output heading.prg"
***
USE "Analysis function.dbf"
DO "Create bargraph.prg"
SET HEADING OFF
***
SCREEN 1 TYPE 0 HEADING "Screen 1" AT 40,2 SIZE 286,492 PIXELS FONT "Geneva",12 COLOR 0,0,0
***
? '
? '          FUNCTIONAL CLASS                CLONES   GENES   GENES   TOTAL   TOTAL   NEW   DIST
? '                                     CLONES   GENES   GENES   GENES   GENES   GENES   GENES
? '
***
*LIST OFF FIELDS P,NAME,CLONES,GENES,NEW,PERCENT,GRAPH,COMPANY
LIST OFF FIELDS P,NAME,CLONES,GENES,NEW,PERCENT,GRAPH
CLOSE DATABASES
ERASE TEMP2.DBF
SET HEADING ON
*USE "SmartGuy:FoxBASE+/Mac:fox files:TEMPMASTER.dbf"
ENDIF
CASE ANAL=8
DO "Subgroup summary 3.prg"
ENDCASE
DO "Test print.prg"
SET PRINT OFF
SET DEVICE TO SCREEN
CLOSE DATABASES
*ERASE TEMPLIB.DBF
*ERASE TEMPNUM.DBF
*ERASE TEMPDESIG.DBF
*ERASE SELECTED.DBF
CLEAR
LOOP
ENDDO

```

```

* COMPRESSION SUBROUTINE FOR ANALYSIS PROGRAMS
USE TEMP1
COUNT TO TOT
REPLACE ALL RFEND WITH 1
MARK1 = 1
SW2=0
DO WHILE SW2=0 ROLL
  IF MARK1 >= TOT
    PACK
    COUNT TO UNIQUE
    COUNT TO NEWGENES FOR D='H'.OR.D='O'
    SW2=1
    LOOP
  ENDIF
GO MARK1
DUP = 1
STORE ENTRY TO TESTA
SW = 0
DO WHILE SW=0 TEST
  SKIP
  STORE ENTRY TO TESTB
  IF TESTA = TESTB
    DELETE
    DUP = DUP+1
    LOOP
  ENDIF
GO MARK1
REPLACE RFEND WITH DUP
MARK1 = MARK1+DUP
SW=1
LOOP
ENDDO TEST
LOOP
ENDDO ROLL
GO TOP
STORE Z TO LOC
USE 'Analysis location.dbf'
LOCATE FOR Z=LOC
REPLACE CLONES WITH TOT
REPLACE GENES WITH UNIQUE
REPLACE NEW WITH NEWGENES
USE TEMP1
SORT ON RFEND/D TO TEMP2
USE TEMP2
?? STR(UNIQUE,5,0)
?? ' genes, for a total of '
?? STR(TOT,5,0)
?? ' .clones'
? '
? ' V Coincidence'
list off fields number,RFEND,L,D,F,Z,R,C,ENTRY,S,DESCRIPTOR,LENGTH,INIT,I

*SET PRINT OFF
CLOSE DATABASES
ERASE TEMP1.DBF
ERASE TEMP2.DBF
USE TEMPDESIG

```

```

* COMPRESSION SUBROUTINE FOR ANALYSIS PROGRAMS
USE TEMP1
COUNT TO TOT
REPLACE ALL RFEND WITH 1
MARK1 = 1
SW2=0
DO WHILE SW2=0 ROLL
  IF MARK1 >= TOT
    PACK
    COUNT TO UNIQUE
    SW2=1
  LOOP
ENDIF
GO MARK1
DUP = 1
STORE ENTRY TO TESTA
SW = 0
DO WHILE SW=0 TEST
  SKIP
  STORE ENTRY TO TESTB
  IF TESTA = TESTB
    DELETE
    DUP = DUP+1
  LOOP
ENDIF
GO MARK1
REPLACE RFEND WITH DUP
MARK1 = MARK1+DUP
SW=1
LOOP
ENDDO TEST
LOOP
ENDDO ROLL
*BROWSE
*SET PRINTER ON
SORT ON DATE TO TEMP2
USE TEMP2
?? STR(UNIQUE,4,0)
?? ' genes, for a total of'
?? STR(TOT,4,0)
?? ' clones'
?
? '          V Coincidence'
COUNT TO P4 FOR I=4
IF P4>0
? STR(P4,3,0)
?? ' genes with priority = 4 (Secondary analysis:)'
list off fields number,RFEND,L,D,F,Z,R,C,ENTRY,S,DESCRIPTOR,LENGTH,INIT for I=4
?
ENDIF
COUNT TO P3 FOR I=3
IF P3>0
? STR(P3,3,0)
?? ' genes with priority = 3 (Full insert sequence:)'
list off fields number,RFEND,L,D,F,Z,R,C,ENTRY,S,DESCRIPTOR,LENGTH,INIT for I=3
?
ENDIF
COUNT TO P2 FOR I=2
IF P2>0
? STR(P2,3,0)
?? ' genes with priority = 2 (Primary analysis complete:)'
list off fields number,RFEND,L,D,F,Z,R,C,ENTRY,S,DESCRIPTOR,LENGTH,INIT for I=2
?
ENDIF
COUNT TO P1 FOR I=1
IF P1>0

```

```
? STR(P1,3,0)
?? ' genes with priority = 1 (Primary analysis needed:)'
list off fields number,RFEND,L,D,P,Z,R,C,ENTRY,S,DESCRIPTOR,LENGTH,INIT for I=1
ENDIF
```

```
*SET PRINT OFF
CLOSE DATABASES
ERASE TEMP1.DBF
ERASE TEMP2.DBF
USE 'SmartGuy\FoxBASE+/Mac:fox files:clones.dbf'
```



```

* COMPRESSION SUBROUTINE FOR ANALYSIS PROGRAMS
USE TEMP1
COUNT TO TOT
REPLACE ALL RFEND WITH 1
MARK1 = 1
SW2=0
DO WHILE SW2=0 ROLL
  IF MARK1 >= TOT
    PACK
    COUNT TO UNIQUE
    SW2=1
  LOOP
ENDIF
GO MARK1
DUP = 1
STORE ENTRY TO TESTA
SW = 0
DO WHILE SW=0 TEST
  SKIP
  STORE ENTRY TO TESTB
  IF TESTA = TESTB
    DELETE
    DUP = DUP+1
  LOOP
ENDIF
GO MARK1
REPLACE RFEND WITH DUP
MARK1 = MARK1+DUP
SW=1
LOOP
ENDDO TEST
LOOP
ENDDO ROLL
*BROWSE
*SET PRINTER ON
SORT ON NUMBER TO TEMP2
USE TEMP2

?? STR(UNIQUE,4,0)
?? ' genes, for a total of '
?? STR(TOT,5,0)
?? ' clones'
? '
      V Coincidence'
list off fields number,RFEND,L,D,F,Z,R,C,ENTRY,S,DESCRIPTOR,LENGTH,INIT,I

*SET PRINT OFF
CLOSE DATABASES
ERASE TEMP1.DBF
ERASE TEMP2.DBF
USE 'SmartGuy\FoxBASE+Mac\fox files\clones.dbf'

```

* COMPRESSION SUBROUTINE FOR ANALYSIS PROGRAMS

USE TEMP1

COUNT TO TOT

REPLACE ALL RFEND WITH 1

MARK1 = 1

SW2=0

DO WHILE SW2=0 ROLL

IF MARK1 >= TOT

PACK

COUNT TO UNIQUE

COUNT TO NEWGENES FOR D='H'.OR.D='O'

SW2=1

LOOP

ENDIF

GO MARK1

DUP = 1

STORE ENTRY TO TESTA

SW = 0

DO WHILE SW=0 TEST

SKIP

STORE ENTRY TO TESTB

IF TESTA = TESTB

DELETE

DUP = DUP+1

LOOP

ENDIF

GO MARK1

REPLACE RFEND WITH DUP

MARK1 = MARK1+DUP

SW=1

LOOP

ENDDO TEST

LOOP

ENDDO ROLL

GO TOP

STORE R TO FUNC

USE "Analysis function.dbf"

LOCATE FOR P=FUNC

REPLACE CLONES WITH TOT

REPLACE GENES WITH UNIQUE

REPLACE NEW WITH NEWGENES.

USE TEMP1

SORT ON RFEND/D TO TEMP2

USE TEMP2

SET HEADING ON

?? STR(UNIQUE,5,0)

?? ' genes, for a total of '

?? STR(TOT,5,0)

?? ' clones'

? ' V Coincidence'

list off fields number,RFEND,L,D,F,Z,R,C,ENTRY,S,DESCRIPTOR,LENGTH,INIT,I

*SCREEN 1 TYPE 0 HEADING "Screen 1" AT 40,2 SIZE 286,492 PIXELS FONT "Geneva",12 COLOR 0,0,

*list off fields RFEND,S,DESCRIPTOR

*SET PRINT OFF

CLOSE DATABASES

ERASE TEMP1.DBF

ERASE TEMP2.DBF

USE TEMPDESIG

```

* COMPRESSION SUBROUTINE FOR ANALYSIS PROGRAMS
USE TEMP1
COUNT TO TOT
REPLACE ALL RFEND WITH 1
MARK1 = 1
SW2=0
DO WHILE SW2=0 ROLL
  IF MARK1 >= TOT
    PACK
    COUNT TO UNIQUE
    SW2=1
  LOOP
ENDIF
GO MARK1
DUP = 1
STORE ENTRY TO TESTA
SW = 0
DO WHILE SW=0 TEST
  SKIP
  STORE ENTRY TO TESTB
  IF TESTA = TESTB
    DELETE
    DUP = DUP+1
  LOOP
ENDIF
GO MARK1
REPLACE RFEND WITH DUP
MARK1 = MARK1+DUP
SW=1
LOOP
ENDDO TEST
LOOP
ENDDO ROLL
GO TOP
STORE F TO DIST
USE "Analysis distribution.dbf"
LOCATE FOR P=DIST
REPLACE CLONES WITH TOT
REPLACE GENES WITH UNIQUE
USE TEMP1
sort on rfend/d to TEMP2
USE TEMP2
?? STR(UNIQUE,5,0)
?? ' genes, for a total of '
?? STR(TOT,5,0)
?? ' clones'
? '
V Coincidence'
list off fields number, RFEND, L, D, F, Z, R, C, ENTRY, S, DESCRIPTOR, LENGTH, INIT, I

*SET PRINT OFF
CLOSE DATABASES
ERASE TEMP1.DBF
ERASE TEMP2.DBF
USE TEMPDESIG

```

```

* COMPRESSION SUBROUTINE FOR ANALYSIS PROGRAMS
USE TEMP1
COUNT TO TOT
REPLACE ALL RFEND WITH 1
MARK1 = 1
SW2=0
DO WHILE SW2=0 ROLL
  IF MARK1 >= TOT
    PACK
    COUNT TO UNIQUE
    SW2=1
  LOOP
ENDIF
GO MARK1
DUP = 1
STORE ENTRY TO TESTA
SW = 0
DO WHILE SW=0 TEST
  SKIP
  STORE ENTRY TO TESTB
  IF TESTA = TESTB
    DELETE
    DUP = DUP+1
  LOOP
ENDIF
GO MARK1
REPLACE RFEND WITH DUP
MARK1 = MARK1+DUP
SW=1
LOOP
ENDDO TEST
LOOP
ENDDO ROLL
GO TOP
USE TEMP1
?? STR(UNIQUE,5,0)
?? ' genes, for a total of '
?? STR(TOT,5,0)
?? ' clones'
? '
                                V Coincidence'
list off fields number, RFEND, L, D, F, Z, R, C, ENTRY, S, DESCRIPTOR, LENGTH, INIT, I

*SET PRINT OFF
CLOSE DATABASES
ERASE TEMP1.DBF
USE TEMPDESIG

```

```

* COMPRESSION SUBROUTINE FOR ANALYSIS PROGRAMS
USE 'SmartGuy:FoxBASE+/Mac:fox files:Clones.dbf'
COPY TO TEMP1 FOR
USE TEMP1
COUNT TO IDGENE FOR D='E'.OR.D='O'.OR.D='H'.OR.D='N'.OR.D='R'.OR.D='A'
DELETE FOR D='N'.OR.D='D'.OR.D='A'.OR.D='U'.OR.D='S'.OR.D='M'.OR.D='R'.OR.D='V'
PACK
COUNT TO TOT
REPLACE ALL RFEND WITH 1
MARK1 = 1
SW2=0
DO WHILE SW2=0 ROLL
  IF MARK1 >= TOT
    PACK
    COUNT TO UNIQUE
    SW2=1
  LOOP
ENDIF
GO MARK1
DUP = 1
STORE ENTRY TO TESTA
SW = 0
DO WHILE SW=0 TEST
  SKIP
  STORE ENTRY TO TESTB
  IF TESTA = TESTB
    DELETE
    DUP = DUP+1
  LOOP
ENDIF
GO MARK1
REPLACE RFEND WITH DUP
MARK1 = MARK1+DUP
SW=1
LOOP
ENDDO TEST
LOOP
ENDDO ROLL
*BROWSE
*SET PRINTER ON
SORT ON RFEND/D,NUMBER TO TEMP2
USE TEMP2
REPLACE ALL START WITH RFEND/IDGENE*10000
?? STR(UNIQUE,5,0)
?? ' genes, for a total of '
?? STR(TOT,5,0)
?? ' clones'
? ' Coincidence V      V Clones/10000'
set heading off
SCREEN 1 TYPE 0 HEADING 'Screen 1' AT 40,2 SIZE 286,492 PIXELS FONT 'Geneva',7 COLOR 0,0,0,
list fields number,RFEND,START,L,D,F,Z,R,C,ENTRY,S,DESCRIPTOR,INIT,I
*SET PRINT OFF
CLOSE DATABASES
ERASE TEMP1.DBF
ERASE TEMP2.DBF
USE 'SmartGuy:FoxBASE+/Mac:fox files:clones.dbf'

```

```

* COMPRESSION SUBROUTINE FOR ANALYSIS PROGRAMS
USE TEMP1
COUNT TO IDGENE FOR D='E'.OR.D='O'.OR.D='H'.OR.D='N'.OR.D='R'.OR.D='A'
DELETE FOR D='N'.OR.D='D'.OR.D='A'.OR.D='U'.OR.D='S'.OR.D='M'.OR.D='R'.OR.D='V'
PACK
COUNT TO TOT
REPLACE ALL RFEND WITH 1
MARK1 = 1
SW2=0
DO WHILE SW2=0 ROLL
  IF MARK1 >= TOT
    PACK
    COUNT TO UNIQUE
    SW2=1
  LOOP
  ENDDO
GO MARK1
DUP = 1
STORE ENTRY TO TESTA
SW = 0
DO WHILE SW=0 TEST
  SKIP
  STORE ENTRY TO TESTB
  IF TESTA = TESTB
    DELETE
    DUP = DUP+1
  LOOP
  ENDDO
GO MARK1
REPLACE RFEND WITH DUP
MARK1 = MARK1+DUP
SW=1
LOOP
ENDDO TEST
LOOP
ENDDO ROLL
*BROWSE
*SET PRINTER ON
SORT ON RFEND/D,NUMBER TO TEMP2
USE TEMP2
REPLACE ALL START WITH RFEND/IDGENE*10000
?? STR(UNIQUE,5,0)
?? ' genes, for a total of '
?? STR(TOT,5,0)
?? ' clones'
? ' Coincidence V      V Clones/10000'
set heading off
SCREEN 1 TYPE 0 HEADING "Screen 1" AT 40,2 SIZE 286,492 PIXELS FONT "Geneva",7 COLOR 0,0,0,
list fields number,RFEND,START,L,D,F,Z,R,C,ENTRY,S,DESCRIPTOR,INIT,I
*SET PRINT OFF
CLOSE DATABASES
ERASE TEMP1.DBF
ERASE TEMP2.DBF
USE "SmartGuy:FoxBASE+/Mac:fox files:clones.dbf"

```



```
USE TEMP1
COUNT TO TOT
?? ' Total of'
?? STR(TOT,4,0)
?? ' clones'
?
*list off fields number,L,D,F,Z,R,C,ENTRY,DESCRIPTOR,LENGTH,RFEND,INIT,I
list off fields number,L,D,F,Z,R,C,ENTRY,DESCRIPTOR
CLOSE DATABASES
ERASE TEMP1.DBF
USE TEMPDESIG
```

```

*Lifescan menu; version 8-7-94
SET TALK OFF
set device to screen
CLEAR
USE "SmartGuy:FoxBASE+/Mac:fox files:clones.dbf"
STORE LUPDATE() TO Update
GO BOTTOM
STORE RECNO() TO cloneno
STORE 6 TO Chooser
DO WHILE .T.
  * Program.: Lifeseq menu.fmt
  * Date....: 1/11/95
  * Version.: FoxBASE+/Mac, revision 1.10
  * Notes....: Format file Lifeseq menu
  *
  SCREEN 1 TYPE 0 HEADING "Screen 1" AT 40,2 SIZE 286,492 PIXELS FONT "Geneva",268 COLOR 0,0,
  @ PIXELS 18,126 TO 77,365 STYLE 28479 COLOR 32767,-25600,-1,-16223,-16721,-15725
  @ PIXELS 110,29 TO 188,217 STYLE 3871 COLOR 0,0,-1,-25600,-1,-1
  @ PIXELS 45,161 SAY "LIFESEQ" STYLE 65536 FONT "Geneva",536 COLOR 0,0,-1,-1,7135,5884
  @ PIXELS 36,269 SAY "TM" STYLE 65536 FONT "Geneva",12 COLOR 0,0,-1,-1,7135,5884
  @ PIXELS 63,143 SAY "Molecular Biology Desktop" STYLE 65536 FONT "Helvetica",18 COLOR 0,0,0,
  @ PIXELS 90,252 TO 251,467 STYLE 28447 COLOR 0,0,-1,-25600,-1,-1
  @ PIXELS 117,270 GET Chooser STYLE 65536 FONT "Chicago",12 PICTURE "@*RV Transcript profiles
  @ PIXELS 135,128 SAY Update STYLE 0 FONT "Geneva",12 SIZE 15,79 COLOR 0,0,0,-25600,-1,-1
  @ PIXELS 171,128 SAY cloneno STYLE 0 FONT "Geneva",12 SIZE 15,79 COLOR 0,0,0,-25600,-1,-1
  @ PIXELS 135,44 SAY "Last update:" STYLE 65536 FONT "Geneva",12 COLOR 0,0,-1,-1,-1,-1
  @ PIXELS 171,44 SAY "Total clones:" STYLE 65536 FONT "Geneva",12 COLOR 0,0,-1,-1,-1,-1
  @ PIXELS 45,296 SAY "v1.30" STYLE 65536 FONT "Geneva",782 COLOR 0,0,-1,-1,-1,-1
  *
  * EOF: Lifeseq menu.fmt
  READ
  DO CASE
  CASE Chooser=1
  DO "SmartGuy:FoxBASE+/Mac:fox files:Output programs:Master analysis 3.prg"
  CASE Chooser=2
  DO "SmartGuy:FoxBASE+/Mac:fox files:Output programs:Subtraction 2.prg"
  CASE Chooser=3
  DO "SmartGuy:FoxBASE+/Mac:fox files:Output programs:Northern (single).prg"
  CASE Chooser=4
  USE "Libraries.dbf"
  BROWSE
  CASE Chooser=5
  DO "SmartGuy:FoxBASE+/Mac:fox files:Output programs:See individual clone.prg"
  CASE Chooser=6
  DO "SmartGuy:FoxBASE+/Mac:fox files:Libraries:Output programs:Menu.prg"
  CASE Chooser=7
  CLEAR
  SCREEN 1 OFF
  RETURN
  ENDCASE

  LOOP
ENDDO

```

```

@1,30 SAY "Database Subset Analysis" STYLE 65536 FONT "Geneva",274 COLOR 0,0,0,-1,-1,-1
?
?
?
?
? date()
?? ' '
?? TIME()
? 'Clone numbers '
?? STR(INITIATE,6,0)
?? ' through '
?? STR(TERMINATE,6,0)
? 'Libraries: '
IF ENTIRE=1
? 'All libraries'
ENDIF
IF ENTIRE=2
MARK=1
DO WHILE .T.
IF MARK>STOPIT
EXIT
ENDIF
USE SELECTED
GO MARK
? ' '
?? TRIM(libname)
STORE MARK+1 TO MARK
LOOP
ENDDO
ENDIF
? 'Designations: '
IF Ematch=0 .AND. Hmatch=0 .AND. Omatch=0
?? 'All'
ENDIF
IF Ematch=1
?? 'Exact,'
ENDIF
IF Hmatch=1
?? 'Human,'
ENDIF
IF Omatch=1
?? 'Other sp.'
ENDIF
IF CONDENSE=1
? 'Condensed format analysis'
ENDIF
IF ANAL=1
? 'Sorted by NUMBER'
ENDIF
IF ANAL=2
? 'Sorted by ENTRY'
ENDIF
IF ANAL=3
? 'Arranged by ABUNDANCE'
ENDIF
IF ANAL=4
? 'Sorted by INTEREST'
ENDIF
IF ANAL=5
? 'Arranged by LOCATION'
ENDIF
IF ANAL=6
? 'Arranged by DISTRIBUTION'
ENDIF
IF ANAL=7
? 'Arranged by FUNCTION'

```

ENDIF

? 'Total clones represented: '

?? STR(STARTOT,6,0)

? 'Total clones analyzed: '

?? STR(ANALTOT,6,0)

?

?

```
USE TEMPl
COUNT TO TOT
?? ' Total of'
?? STR(TOT,4,0)
?? ' clones'
?
*list off fields number,L,D,F,Z,R,C,ENTRY,DESCRIPTOR,LENGTH,RFEND,INIT,I
list off fields number,L,D,F,Z,R,C,ENTRY,DESCRIPTOR
CLOSE DATABASES
ERASE TEMPl.DBF
USE TEMPDESIG
```

```
USE TEMP1
COUNT TO TOT
?? ' Total of'
?? STR(TOT,4,0)
?? ' clones'
?
*list off fields number,L,D,F,Z,R,C,ENTRY,DESCRIPTOR,LENGTH,RFEND,INIT,I
list off fields number,L,D,F,Z,R,C,ENTRY,DESCRIPTOR
CLOSE DATABASES
ERASE TEMP1.DBF
USE TEMPOESIG
```

```

*Northern (single), version 11-25-94
close databases
SET TALK OFF
SET PRINT OFF
SET EXACT OFF
CLEAR
STORE ' ' TO Eobject
STORE ' ' TO Dobject
STORE 0 TO Numb
STORE 0 TO Zog
STORE 1 TO Bail
DO WHILE .T.
* Program.: Northern (single).fmt
* Date.....: 8/ 8/94
* Version.: FoxBASE+/Mac, revision 1.10
* Notes.....: Format file Northern (single)
*
SCREEN 1 TYPE 0 HEADING "Screen 1" AT 40,2 SIZE 286,492 PIXELS FONT "Geneva",12 COLOR 0,0,0
@ PIXELS 15,81 TO 45,397 STYLE 28447 COLOR 0,0,-1,-25600,-1,-1
@ PIXELS 89,79 TO 192,422 STYLE 28447 COLOR 0,0,0,-25600,-1,-1
@ PIXELS 115,98 SAY "Entry #:" STYLE 65536 FONT "Geneva",12 COLOR 0,0,0,-1,-1,-1
@ PIXELS 115,173 GET Eobject STYLE 0 FONT "Geneva",12 SIZE 15,142 COLOR 0,0,0,-1,-1,-1
@ PIXELS 145,89 SAY "Description" STYLE 65536 FONT "Geneva",12 COLOR 0,0,0,-1,-1,-1
@ PIXELS 145,173 GET Dobject STYLE 0 FONT "Geneva",12 SIZE 15,241 COLOR 0,0,0,-1,-1,-1
@ PIXELS 35,89 SAY "Single Northern search screen" STYLE 65536 FONT "Geneva",274 COLOR 0,0,-
@ PIXELS 220,162 GET Bail STYLE 65536 FONT "Chicago",12 PICTURE "@*R Continue;Bail out" SIZE
@ PIXELS 175,98 SAY "Clone #:" STYLE 65536 FONT "Geneva",12 COLOR 0,0,0,-1,-1,-1
@ PIXELS 175,173 GET Numb STYLE 0 FONT "Geneva",12 SIZE 15,70 COLOR 0,0,0,-1,-1,-1
@ PIXELS 80,152 SAY "Enter any ONE of the following:" STYLE 65536 FONT "Geneva",12 COLOR -1,
*
* EOF: Northern (single).fmt
READ
IF Bail=2
CLEAR
screen 1 off
RETURN
ENDIF
USE "SmartGuy:FoxBASE+/Mac:Fox files:Lookup.dbf"
SET TALK ON

IF Eobject<>'
STORE UPPER(Eobject) to Eobject
SET SAFETY OFF
SORT ON Entry TO "Lookup entry.dbf"
SET SAFETY ON
USE "Lookup entry.dbf"
LOCATE FOR Look=Eobject
IF .NOT.FOUND()
CLEAR
LOOP
ENDIF
BROWSE
STORE Entry TO Searchval
CLOSE DATABASES
ERASE "Lookup entry.dbf"
ENDIF

IF Dobject<>'
SET EXACT OFF
SET SAFETY OFF
SORT ON descriptor TO "Lookup descriptor.dbf"
SET SAFETY On
USE "Lookup descriptor.dbf"
LOCATE FOR UPPER(TRIM(descriptor))=UPPER(TRIM(Dobject))
IF .NOT.FOUND()
CLEAR

```



```

LOOP
ENDIF
BROWSE
STORE Entry TO Searchval
CLOSE DATABASES
ERASE "Lookup descriptor.dbf"
SET EXACT ON
ENDIF

IF Numb<>0
USE "SmartGuy:FoxBASE+/Mac:Fox files:clones.dbf"
GO Numb
BROWSE
STORE Entry TO Searchval
ENDIF

CLEAR
? 'Northern analysis for entry '
?? Searchval
?
? 'Enter Y to proceed'
WAIT TO OK
CLEAR
IF UPPER(OK) <> 'Y'
screen 1 off
RETURN
ENDIF

* COMPRESSION SUBROUTINE FOR Library.dbf
? 'Compressing the Libraries file now...'
USE "SmartGuy:FoxBASE+/Mac:Fox files:libraries.dbf"
SET SAFETY OFF
SORT ON library TO "Compressed libraries.dbf"
* FOR entered>0
SET SAFETY ON
USE "Compressed libraries.dbf"
DELETE FOR entered=0
PACK
COUNT TO TOT
MARK1 = 1
SW2=0
DO WHILE SW2=0 ROLL
  IF MARK1 >= TOT
    PACK
    SW2=1
  LOOP
  ENDIF
GO MARK1
STORE library TO TESTA
SKIP
STORE Library TO TESTB
IF TESTA = TESTB
DELETE
ENDIF
MARK1 = MARK1+1
LOOP
ENDDO ROLL

* Northern analysis
CLEAR
? 'Doing the northern now...'
SET TALK ON
USE "SmartGuy:FoxBASE+/Mac:Fox files:clones.dbf"
SET SAFETY OFF
COPY TO "Hits.dbf" FOR entry=searchval
SET SAFETY ON

```

```

CLOSE DATABASES
SELECT 1
USE "Compressed libraries.dbf"
STORE RECCOUNT() TO Entries
SELECT 2
USE "Hits.dbf"
Mark=1
DO WHILE .T.
  SELECT 1
  IF Mark>Entries
    EXIT
  ENDF
  GO MARK
  STORE library TO Jigger
  SELECT 2
  COUNT TO Zog FOR library=Jigger
  SELECT 1
  REPLACE hits with Zog
  Mark=Mark+1
  LOOP
ENDDO

SELECT 1
BROWSE FIELDS LIBRARY,LIENAME,ENTERED,HITS AT 0,0
CLEAR
? 'Enter Y to print:'
WAIT TO PRINSET
IF UPPER(PRINSET)='Y'
  SET PRINT ON
  CLEAR
  EJECT
  SCREEN 1 TYPE 0 HEADING "Screen 1" AT 40,2 SIZE 286,492 PIXELS FONT "Geneva",14 COLOR 0,0,0
  ? 'DATABASE ENTRIES MATCHING ENTRY '
  ?? Searchval
  ? DATE()
  ?
  SCREEN 1 TYPE 0 HEADING "Screen 1" AT 40,2 SIZE 286,492 PIXELS FONT "Geneva",7 COLOR 0,0,0,
  LIST OFF FIELDS library,libname,entered,hits
  ?
  ?
  SELECT 2
  LIST OFF FIELDS NUMBER,LIBRARY,D,S,F,Z,R,ENTRY,DESCRIPTOR,RESTART,START,RFEND
  SET TALK OFF
  SET PRINT OFF
  ENDF
  CLOSE DATABASES
  SET TALK OFF
  CLEAR
  DO "Test print.prg"
  RETURN

```

TABLE 6

library	libname
ADENINB01	Inflamed adenoid
ADRENOR01	Adrenal gland (r)
ADRENOT01	Adrenal gland (T)
AMLBNOT01	AML blast cells (T)
BMARNOT01	Bone marrow
BMARNOT02	Bone marrow (T)
CARDNOT01	Cardiac muscle (T)
CHAONOT01	Chin. hamster ovary
CORNNOT01	Corneal stroma
FIBRAGT01	Fibroblast, AT 5
FIBRAGT02	Fibroblast, AT 30
FIBRANT01	Fibroblast, AT
FIBRNGT01	Fibroblast, uv 5
FIBRNGT02	Fibroblast, uv 30
FIBRNOT01	Fibroblast
FIBRNOT02	Fibroblast, normal
HMC1NOT01	Mast cell line HMC-1
HUVELPB01	HUVEC IFN,TNF,LPS
HUVENOB01	HUVEC control
HUVSTB01	HUVEC shear stress
HYPONOB01	Hypothalamus
KIDNOT01	Kidney (T)
LIVRNOT01	Liver (T)
LUNGNOT01	Lung (T)
MUSCNOT01	Skeletal muscle (T)
OVIDNOB01	Oviduct
PANCNOT01	Pancreas, normal
PITUNOR01	Pituitary (r)
PITUNOT01	Pituitary (T)
PLACNOB01	Placenta
SINTNOT02	Small intestine (T)
SPLNFET01	Spleen+liver, fetal
SPLNNOT02	Spleen (T)
STOMNOT01	Stomach
SYNORAE01	Rheum. synovium
TBLYNOT01	T + B lymphoblast
TESTNOT01	Testis (T)
THP1NOB01	THP-1 control
THP1PEB01	THP phorbol
THP1PLB01	THP-1 phorbol LPS
U937NOT01	U937, monocytic leuk

number	library	d s f z r entry	descriptor	rfstart	rfstart1	rfend
2304	U937NOT01	E H C C T HUMEF1B	Elongation factor 1-beta	0	0	773
3240	HMC1NOT01	E H C C T HUMEF1B	Elongation factor 1-beta	0	370	773
3269	HMC1NOT01	E H C C T HUMEF1B	Elongation factor 1-beta	0	371	773
4693	HMC1NOT01	E H C C T HUMEF1B	Elongation factor 1-beta	0	470	773
8989	HMC1NOT01	E H C C T HUMEF1B	Elongation factor 1-beta	0	327	773
9139	HMC1NOT01	E H C C T HUMEF1B	Elongation factor 1-beta	0	375	773

WHAT IS CLAIMED IS:

1. A method of analyzing a specimen containing gene transcripts, said method comprising the steps of:
 - (a) producing a library of biological sequences;
 - 5 (b) generating a set of transcript sequences, where each of the transcript sequences in said set is indicative of a different one of the biological sequences of the library;
 - (c) processing the transcript sequences in a
10 programmed computer in which a database of reference transcript sequences indicative of reference biological sequences is stored, to generate an identified sequence value for each of the transcript sequences, where each said identified sequence value is indicative of a sequence
15 annotation and a degree of match between one of the transcript sequences and at least one of the reference transcript sequences; and
 - (d) processing each said identified sequence value to generate final data values indicative of a number of times
20 each identified sequence value is present in the library.
2. The method of claim 1, wherein step (a) includes the steps of:
 - obtaining a mixture of mRNA;
 - making cDNA copies of the mRNA;
 - 25 isolating a representative population of clones transfected with the cDNA and producing therefrom the library of biological sequences.
3. The method of claim 1, wherein the biological sequences are cDNA sequences.
- 30 4. The method of claim 1, wherein the biological sequences are RNA sequences.
5. The method of claim 1, wherein the biological sequences are protein sequences.

6. The method of claim 1, wherein a first value of said degree of match is indicative of an exact match, and a second value of said degree of match is indicative of a non-exact match.

- 5 7. A method of comparing two specimens containing gene transcripts, said method comprising:
- (a) analyzing a first specimen according to the method of claim 1;
 - (b) producing a second library of biological
10 sequences;
 - (c) generating a second set of transcript sequences, where each of the transcript sequences in said second set is indicative of a different one of the biological sequences of the second library;
 - 15 (d) processing the second set of transcript sequences in said programmed computer to generate a second set of identified sequence values known as further identified sequence values, where each of the further identified sequence values is indicative of a sequence annotation and
20 a degree of match between one of the biological sequences of the second library and at least one of the reference sequences;
 - (e) processing each said further identified sequence value to generate further final data values indicative of a
25 number of times each further identified sequence value is present in the second library; and
 - (f) processing the final data values from the first specimen and the further identified sequence values from the second specimen to generate ratios of transcript
30 sequences, each of said ratio values indicative of differences in numbers of gene transcripts between the two specimens.

8. A method of quantifying relative abundance of mRNA in a biological specimen, said method comprising the steps
35 of:

- (a) isolating a population of mRNA transcripts from the biological specimen;

(b) identifying genes from which the mRNA was transcribed by a sequence-specific method;

(c) determining numbers of mRNA transcripts corresponding to each of the genes; and

5 (d) using the mRNA transcript numbers to determine the relative abundance of mRNA transcripts within the population of mRNA transcripts.

9. A diagnostic method which comprises producing a gene transcript image, said method comprising the steps of:

10 (a) isolating a population of mRNA transcripts from a biological specimen;

(b) identifying genes from which the mRNA was transcribed by a sequence-specific method;

(c) determining numbers of mRNA transcripts
15 corresponding to each of the genes; and

(d) using the mRNA transcript numbers to determine the relative abundance of mRNA transcripts within the population of mRNA transcripts, where data determining the relative abundance values of mRNA transcripts is the gene
20 transcript image of the biological specimen.

10. The method of claim 9, further comprising:

(e) providing a set of standard normal and diseased gene transcript images; and

(f) comparing the gene transcript image of the
25 biological specimen with the gene transcript images of step (e) to identify at least one of the standard gene transcript images which most closely approximate the gene transcript image of the biological specimen.

11. The method of claim 9, wherein the biological
30 specimen is biopsy tissue, sputum, blood or urine.

12. A method of producing a gene transcript image, said method comprising the steps of

(a) obtaining a mixture of mRNA;

(b) making cDNA copies of the mRNA;

- (c) inserting the cDNA into a suitable vector and using said vector to transfect suitable host strain cells which are plated out and permitted to grow into clones, each clone representing a unique mRNA;
- 5 (d) isolating a representative population of recombinant clones;
- (e) identifying amplified cDNAs from each clone in the population by a sequence-specific method which identifies gene from which the unique mRNA was transcribed;
- 10 (f) determining a number of times each gene is represented within the population of clones as an indication of relative abundance; and
- (g) listing the genes and their relative abundance in order of abundance, thereby producing the gene transcript
- 15 image.

13. The method of claim 12, also including the step of diagnosing disease by:

- repeating steps (a) through (g) on biological specimens from random sample of normal and diseased humans,
- 20 encompassing a variety of diseases, to produce reference sets of normal and diseased gene transcript images;
- obtaining a test specimen from a human, and producing a test gene transcript image by performing steps (a) through (g) on said test specimen;
- 25 comparing the test gene transcript image with the reference sets of gene transcript images; and
- identifying at least one of the reference gene transcript images which most closely approximates the test gene transcript image.

30 14. A computer system for analyzing a library of biological sequences, said system including:

- means for receiving a set of transcript sequences, where each of the transcript sequences is indicative of a different one of the biological sequences of the library;
- 35 and
- means for processing the transcript sequences in the computer system in which a database of reference transcript

sequences indicative of reference biological sequences is stored, wherein the computer is programmed with software for generating an identified sequence value for each of the transcript sequences, where each said identified sequence
5 value is indicative of a sequence annotation and a degree of match between a different one of the biological sequences of the library and at least one of the reference transcript sequences, and for processing each said identified sequence value to generate final data values
10 indicative of a number of times each identified sequence value is present in the library.

15. The system of claim 14, also including:

library generation means for producing the library of biological sequences and generating said set of transcript
15 sequences from said library.

16. The system of claim 15, wherein the library generation means includes:

means for obtaining a mixture of mRNA;
means for making cDNA copies of the mRNA;
20 means for inserting the cDNA copies into cells and permitting the cells to grow into clones;
means for isolating a representative population of the clones and producing therefrom the library of biological sequences.

SYBASE database Structure

Library Preparation

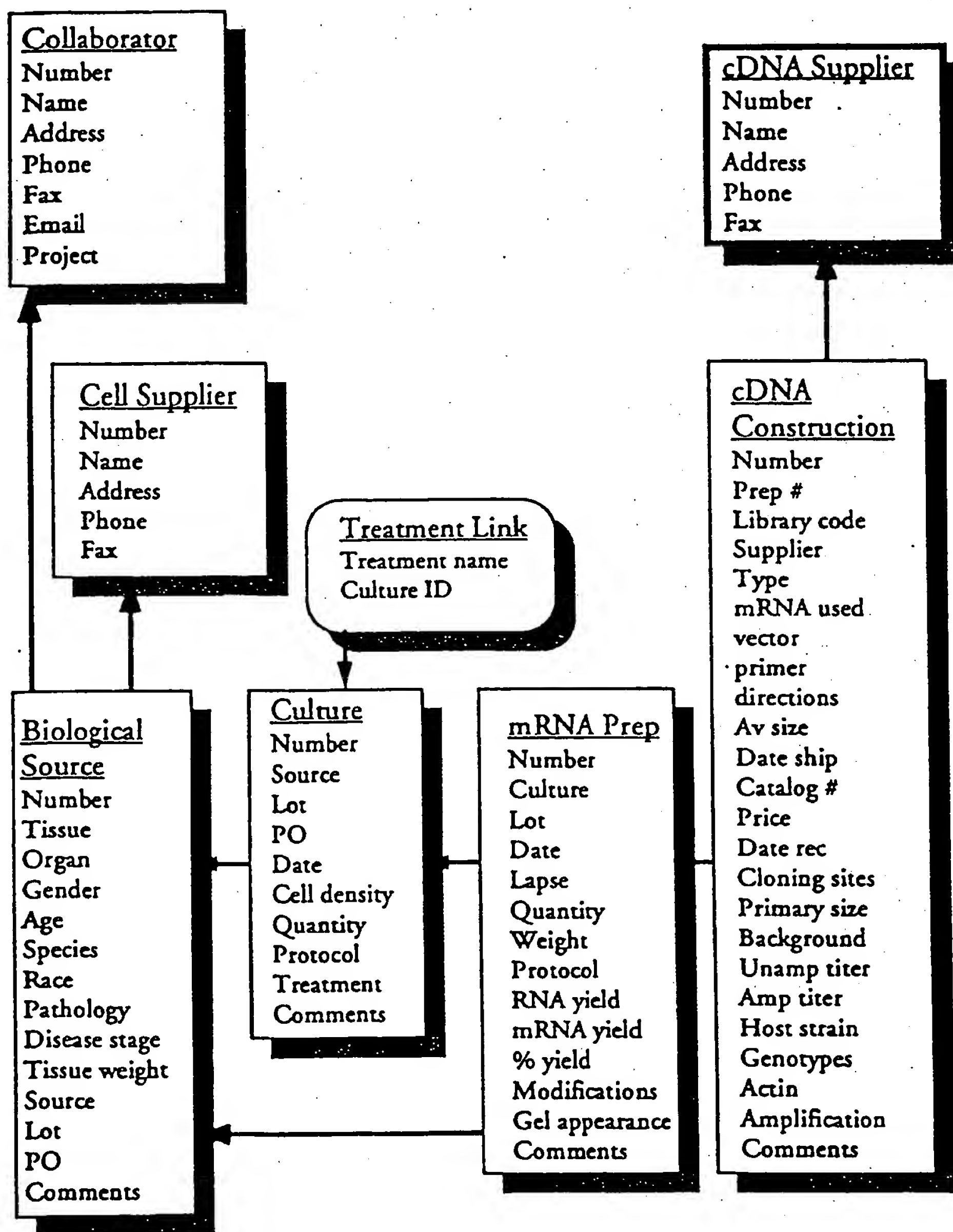


Figure 1

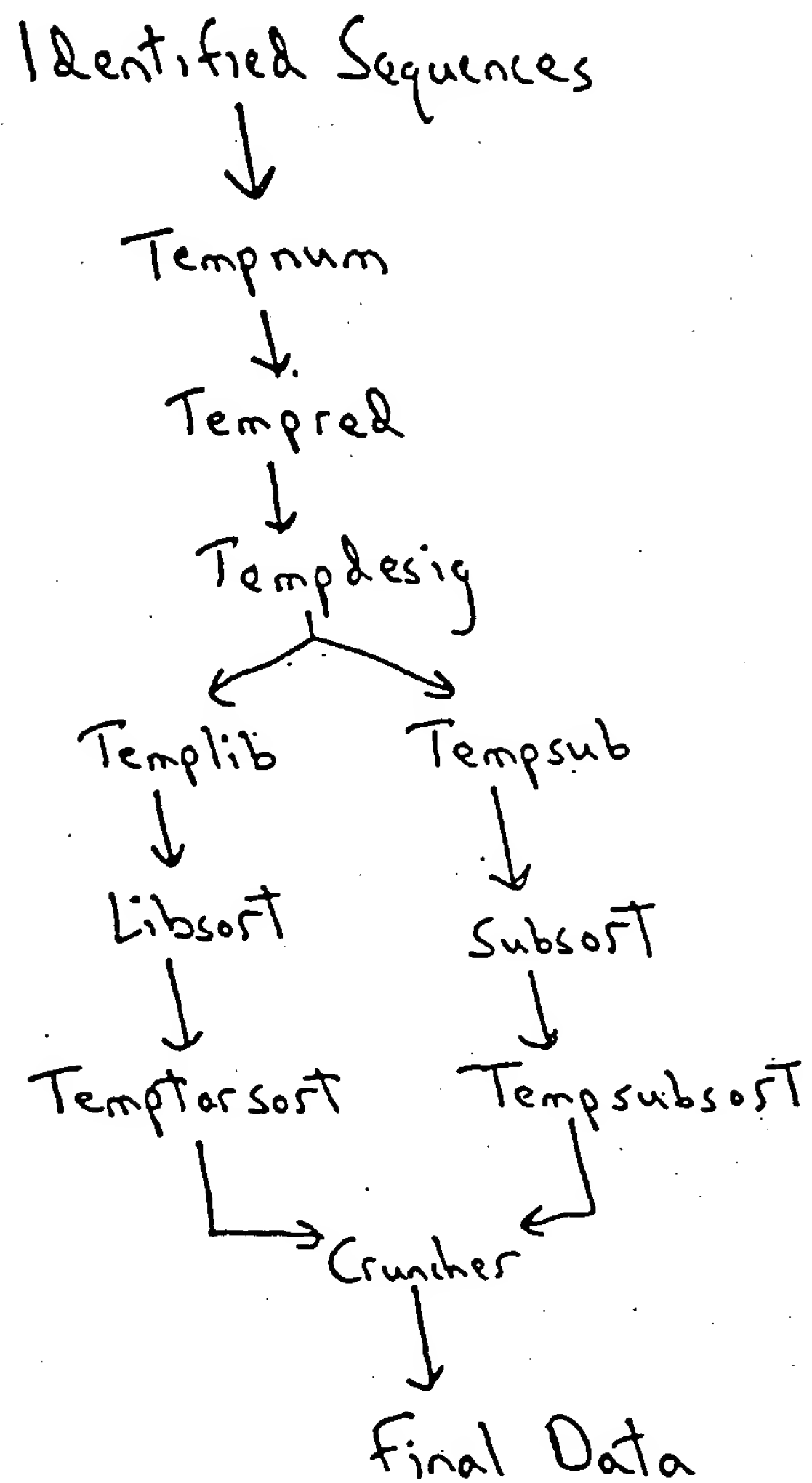


Figure 2

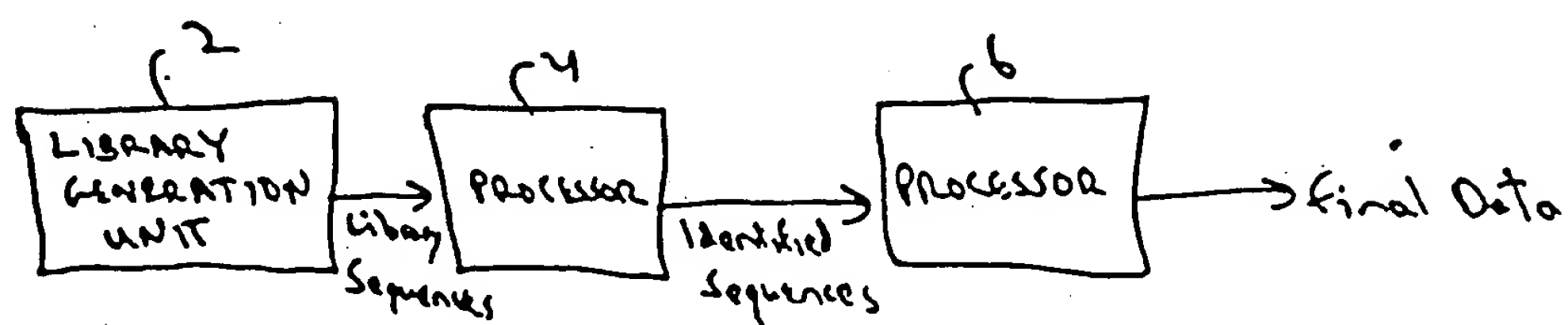


Figure 3

Incyte Bioinformatics Process

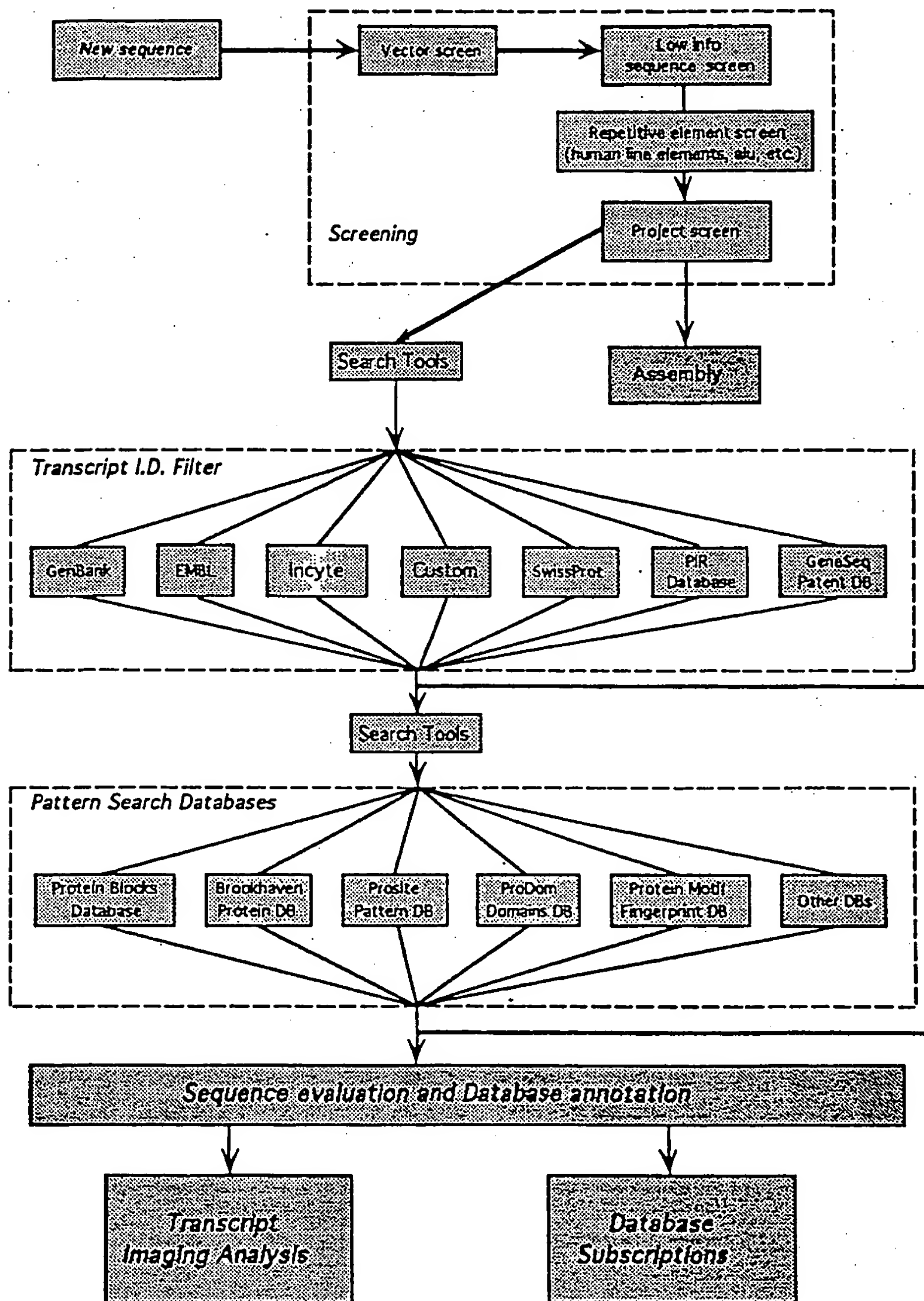


Figure 4

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/01160

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : C12Q 1/68; G06F 15/00

US CL : 435/6; 364/413.02

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/6; 364/413.02

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CAS ONLINE, APS, transcript, transcripts, cdan#, mrna#, frequenc?, distribut?, abundanc?

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	IntelliGenetics Suite, Release 5.4, Advanced Training Manual, issued January 1993 by IntelliGenetics, Inc. 700 East El Camino Real, Mountain View, California 94040, United States of America, pages (1-6)-(1-19) and (2-9)-(2-14), see entire document.	15 and 16 ----- 1-14
Y	Science, Volume 252, issued 21 June 1991, M.D. Adams et al, "Complementary DNA sequencing: Expressed sequence tags and human genome project", pages 1651-1656, see entire document.	1-16

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	*T	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
A document defining the general state of the art which is not considered to be of particular relevance	*X*	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
E earlier document published on or after the international filing date	*Y*	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*G*	document member of the same patent family
O document referring to an oral disclosure, use, exhibition or other means		
P document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

27 APRIL 1995

Date of mailing of the international search report

04 MAY 1995

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US95/01160

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Nucleic Acids Research, Volume 19, No. 25, issued 1991, E. Hara et al, "Subtractive cDNA cloning using oligo(dT) ₃₀ -latex and PCR: isolation of cDNA clones specific to undifferentiated human embryonal carcinoma cells", pages 7097-7104, see entire document.	1-16
X — Y	Nature Genetics, Volume 2, No. 3, issued November 1992, K. Okubo et al, "Large scale cDNA sequencing for analysis of quantitative and qualitative aspects of gene expression", pages 173-179, see narrative text portion of entire document.	1, 3 ----- 2 and 4-16